AD-A094 903 ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT --ETC F/G 6/13
PHOTOBACTERIAL RESPONSE TO CADMIUM CHLORIDE, MERCURIC CHLORIDE,--ETC(U)
JUL 80 R N SHIOTSUKA, P H GIBBS, S ALLONGO
UNCLASSIFIED USAMBRDL-TR-8012 NL

1 OF 1
AD
A094903

END
DATE
FILMED
3 81
DTIC

LEVEL

AD

10

Technical Report 8012

AD A094903

PHOTOBACTERIAL RESPONSE TO CADMIUM CHLORIDE, MERCURIC CHLORIDE, AND SELENIUM DIOXIDE: DOSE-RESPONSE AND INTERACTION STUDIES

RONALD N. SHIOTSUKA
PAUL H. GIBBS
SUSANA ALLONGO

US ARMY MEDICAL BIOENGINEERING RESEARCH and DEVELOPMENT LABORATORY
Fort Detrick
Frederick, MD 21701

JULY 1980

APPROVED FOR PUBLIC RELEASE;
DISTRIBUTION UNLIMITED

US ARMY MEDICAL RESEARCH and DEVELOPMENT COMMAND
Fort Detrick
Frederick, MD 21701

NOTICE

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: TECHNICAL REPORT 8012	2. GOVT ACCESSION NO.: AD-A094 903
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): PHOTOBACTERIAL RESPONSE TO CADMIUM CHLORIDE, MERCURIC CHLORIDE, AND SELENIUM DIOXIDE: DOSE-RESPONSE AND INTERACTION STUDIES	5. TYPE OF REPORT & PERIOD COVERED: Technical Report November 1978-December 1979
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Ronald N. Shiotsuka, Paul H. Gibbs, Susana Allongo	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: U.S. Army Medical Bioengineering Research and Development Laboratory, ATTN: SGRD-UBG, Fort Detrick, Frederick, MD 21701	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 61101A 3A161101A91C/00/306
11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Medical Research and Development Command, ATTN: SGRD-RMS, Fort Detrick, Frederick, MD 21701	12. REPORT DATE: July 1980
	13. NUMBER OF PAGES: 87
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Beneckea harveyi, Bioluminescence, Cadmium chloride, Mercuric chloride, Photobacteria, Selenium dioxide, Toxicity

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

An ideal biologic toxicity screening system would have the following characteristics: (1) test organisms are simple to maintain, (2) organisms should possess a reproducible and easily measured endpoint, (3) simultaneous testing of a large number of individuals is permitted, (4) screening of several chemicals during each screening test is permitted, (5) the duration of each screening test is of reasonable length, and (6) the response of the organisms is dose dependent. The photobacterial toxicity screening system

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. ABSTRACT (Cont.)

used in this study meets these criteria: the photobacteria are easily grown in a liquid medium and require subculturing once daily; their luminescent response is reproducible and accurately measured by a photometer; a large number of chemicals may be screened during an 8- to 9-hour span; and the response of the photobacterial bioluminescent activity is dose dependent to at least one group of compounds tested—nitrotoluenes.

The objective of this study was to determine the dose-response characteristics of the photobacterial toxicity screening system (PTSS) when challenged by cadmium chloride, mercuric chloride, and selenium dioxide in aqueous solutions and to determine the response of the PTSS to various combinations of these three compounds.

Dose-response studies of the three compounds tested separately indicated that mercuric chloride was the most potent in reducing the bioluminescent activity of the photobacteria, selenium dioxide slightly less potent, and cadmium chloride the least potent. These conclusions are based on the differences in median effective concentration (EC_{50}) of the dose-response studies.

The next sequence of studies examined interactions of the three compounds. The test strategy involved fixing the concentration of one compound at its EC_{10} (10 percent reduction in luminescent activity) and producing a dose-response curve by adding different concentrations of the second compound. The EC_{50} values from such paired compounds studies were compared with the results of the EC_{50} values from single compound studies. The studies indicated that the results from the photobacterial test system were consistent with those reported in the literature for the combinations used with two exceptions: our inability to demonstrate a statistically significant effect of selenium dioxide on the EC_{50} of mercuric chloride and the inability of mercuric chloride to influence the cadmium chloride EC_{50}. All other combinations resulted in a protective effect by the compound presented at its EC_{10} concentration.

The results of this study clearly indicate that the response of the PTSS to the three compounds tested is dose dependent and that the PTSS promises to be a viable screening system for interaction testing of chemicals. In order to validate the latter capability, more compounds need to be tested and at different concentrations of each to supplement the data presented here on fixed low (EC_{10}) concentrations of one of the pair of compounds.

SUMMARY

Dose-response studies were first conducted separately on three chemicals. These studies indicated that mercuric chloride was the most potent, followed by selenium dioxide and then by cadmium chloride. Various paired combinations of the three metals were tested to determine the nature of the response of the photobacterial test system to combinations of the toxic chemicals. The test strategy involved fixing the dose of the first metal at its EC_{10} concentration and varying the concentration of the second to produce a dose-response curve. The EC_{50} values from the dose-response curves of studies involving different pairs of metals were compared to determine the nature of the response. It was observed that cadmium chloride caused a reduction in the potency of mercuric chloride and selenium dioxide. Selenium dioxide reduced the potency of cadmium chloride but not of mercuric chloride. Mercuric chloride reduced the potency of selenium dioxide but not of cadmium chloride. Selenium dioxide did not affect the potency of mercuric chloride, and mercuric chloride did not change the potency of cadmium chloride. The latter two findings are equivocal because of the large variances. Further studies are in progress.

TABLE OF CONTENTS

	Page
SUMMARY	1
LIST OF FIGURES	4
LIST OF TABLES	5
INTRODUCTION	7
MATERIALS AND METHODS	8
RESULTS	9
Single Metal Studies	9
Paired Metal Studies	14
DISCUSSION	22
REFERENCES	25
APPENDIX A: Raw Data from Single Test Chemical Studies	27
APPENDIX B: Converted Data from Single Test Chemical Studies	37
APPENDIX C: Raw Data from Combination of Test Chemical Studies	47
APPENDIX D: Converted Data from Combination of Test Chemical Studies	66

LIST OF FIGURES

		Page
1.	Bioluminescent Response of *B. harveyi* to Selenium Dioxide	10
2.	Bioluminescent Response of *B. harveyi* to Mercuric Chloride	11
3.	Bioluminescent Response of *B. harveyi* to Cadmium Chloride	12
4.	Response of *B. harveyi* to a Fixed Concentration of Mercuric Chloride (0.08 mg/L) and Different Concentrations of Selenium Dioxide	15
5.	Response of *B. harveyi* to a Fixed Concentration of Mercuric Chloride (0.08 mg/L) and Different Concentrations of Cadmium Chloride	16
6.	Response of *B. harveyi* to a Fixed Concentration of Cadmium Chloride (0.48 mg/L) and Different Concentrations of Selenium Dioxide	17
7.	Response of *B. harveyi* to a Fixed Concentration of Selenium Dioxide (0.11 mg/L) and Different Concentrations of Mercuric Chloride	18
8.	Response of *B. harveyi* to a Fixed Concentration of Cadmium Chloride (0.48 mg/L) and Different Concentrations of Mercuric Chloride	19
9.	Response of *B. harveyi* to a Fixed Concentration of Selenium Dioxide (0.11 mg/L) and Different Concentrations of Cadmium Chloride	20

LIST OF TABLES

Page

1. Paired Combinations of Three Metals 9

2. Estimates of the EC_{50} for Single Metal Studies 13

3. Differences in the Mean EC_{50} Values of Three Metals 14

4. Linear Regression Analyses of Combination Studies for Three Metals 21

5. Summary of Mean EC_{50} Values and Comparison of Response by T-test 21

INTRODUCTION

Complex interactions of chemicals with biologic systems are assumed to occur but are infrequently studied in detail. Occupational and environmental health hazards are characterized by the simultaneous exposure of the target population to a number of potentially toxic chemicals. More realistic "safe-exposure" levels than those available today can only be established by a comprehensive integration of the many environmental factors affecting the human population.

One of the major reasons why interaction testing of toxic chemicals is not conducted routinely is that such endeavors are cost prohibitive under most circumstances. For example, current health-effects testing strategies and procedures are usually cost and time prohibitive when more than one chemical is tested. Thus, Muul et al.[1] have recently expressed the need for a battery of short-term screening tests that are predictive of long-term toxic effects.

A need for short-term tests to screen for mutagenesis and carcinogenesis has led to the development, validation, and general acceptance by the scientific community of a diverse group of tests using bacteria, mammalian cells in tissue culture, and whole animals. Less effort is evident in the development of short-term tests predictive of toxicity other than mutagenesis and carcinogenesis. A battery of short-term tests predictive of chronic effects may be a cost-effective approach to the assessment of chemical interactions. The use of a battery of short-term tests to screen a large number of chemicals for their individual toxicity and for their interaction(s) may then provide a "filtering" mechanism such that a chemical or a specific combination of chemicals that appears to pose the greatest health hazard may be identified for more comprehensive evaluation in whole animal subchronic and chronic exposure studies. Such a testing strategy may culminate in epidemiologic studies of incidental or occupational human exposures and medical surveillance programs.

An earlier report by Shiotsuka et al.[2] described the development of a photobacterial toxicity screening system. Seven nitrotoluenes and two nitrobenzonitriles were tested. Additional work to determine the nature of response of the photobacterial test system to another class of chemicals, the metals, and the test system's response to paired combinations of three metals is the subject of this second report. Criteria for selection of the test chemicals were that they demonstrate adequate solubility in distilled water at approximately 27°C and that the chemicals when solubilized not produce a colored or otherwise opaque solution. An opaque solution may attenuate the transmission of light and thus cause inaccuracies in the measurement of photobacterial luminescence. One drawback of the earlier study (Shiotsuka et al.[2]) was the low level of solubility of the nitro-organics in the aqueous photobacterial culture medium, thus requiring the addition of acetone. Possible interactions of acetone and the test chemicals and their combined impact on the photobacterial system were acknowledged, but the scope of that

effort did not permit investigation of these problems. Thus, for this study, the chemicals selected for testing are mercuric chloride, cadmium chloride, and selenium dioxide. These chemicals are readily soluble in distilled water and they also meet the second requirement--that of not producing a colored or otherwise opaque solution.

There are numerous reviews of the toxicity of the three metals used in this study and they will not be reiterated in this report. There are far fewer reports on interactions of these metals, and these interaction studies have been recently reviewed by Parizek,[3] Berlin,[4] Magos,[5] Parizek,[6] and Groth et al.[7]

MATERIALS AND METHODS

Beneckea harveyi from the American Type Culture Collection (No. 14126) was grown in a sterilized liquid medium. Preparation of the culture medium, daily transfer of the stock bacterial culture, and the culture conditions were described in an earlier report by Shiotsuka et al.[2]

Each of the three test chemicals was dissolved in distilled water. The appropriate dilutions were made such that a constant volume (10 µl) was delivered to each test tube regardless of the concentration. All concentrations are reported as nominal values and not measured values in this study. The small volumes in the test tubes did not permit the analysis of the final concentration of the metals.

The specific testing procedure used in this study involved preparing six replicates per dose for each chemical or combination of chemicals tested. For the single chemical studies, 10 µl of the test or control solution followed by 100 µl of the photobacterial test solution were added to each of the six replicate test tubes. The strategy for interaction testing involved fixing the concentration of one metal at approximately its EC_{10} and varying the concentration of the second metal to produce a dose-response curve. The six possible combinations of the three metals are shown in Table 1. The control group consisted of 10 µl of distilled water and 100 µl of the photobacterial test solution. The photobacterial test solution consisted of an aliquot of the 24-hour stock bacterial culture diluted with fresh culture medium to an optical density of 0.046 at a wavelength of 620 nm using a Spectronic 20 (Bausch & Lomb).* During incubation, one test tube from the control group was continuously monitored, and when its luminescent activity reached a peak, all

*Mention of a proprietary product is for identification purposes only and does not imply endorsement by the Department of the Army or Department of Defense.

TABLE 1. PAIRED COMBINATIONS OF THREE METALS

Varied Concentrations (Range in mg/L)	Fixed Concentration (mg/L)
Mercuric Chloride (0.25 to 1.00)	Cadmium Chloride (0.48)
Mercuric Chloride (0.25 to 1.00)	Selenium Dioxide (0.11)
Selenium Dioxide (2.45 to 82.11)	Cadmium Chloride (0.48)
Selenium Dioxide (4.13 to 82.11)	Mercuric Chloride (0.08)
Cadmium Chloride (1.67 to 83.35)	Mercuric Chloride (0.08)
Cadmium Chloride (5.00 to 116.69)	Selenium Dioxide (0.11)

other test tubes were read in the photometer. A Chem-Glow Photometer (American Instrument Co.)* was used for all bioluminescence measurements. It was used at its least sensitive setting, which provided a working range of 0 to 100 units of luminescence. An electronic digital thermometer (Cole-Palmer)* and a dual channel strip chart recorder (Cole-Palmer)* were used for continuous measurement and recording of the incubator temperature.

RESULTS

Three separate dose-response studies were conducted on each chemical and on each combination of chemicals. The raw data (bioluminescence values) from the single test chemical studies are shown in Appendix A. The raw data were converted using the following equation:

$$\text{Bioluminescence} = 100 - [(\text{raw data}/\bar{x} \text{ of control group}) \times 100]$$

The converted data are shown in Appendix B. The raw data from the combination of test chemical studies are given in Appendix C and the converted data in Appendix D. The converted values were then plotted on semi-logarithmic paper with the dose on the log scale.

Single Metal Studies

The results of the three dose-response studies for selenium dioxide, mercuric chloride, and cadmium chloride are shown in Figures 1, 2, and 3,

*Mention of a proprietary product is for identification purposes only and does not imply endorsement by the Department of the Army or Department of Defense.

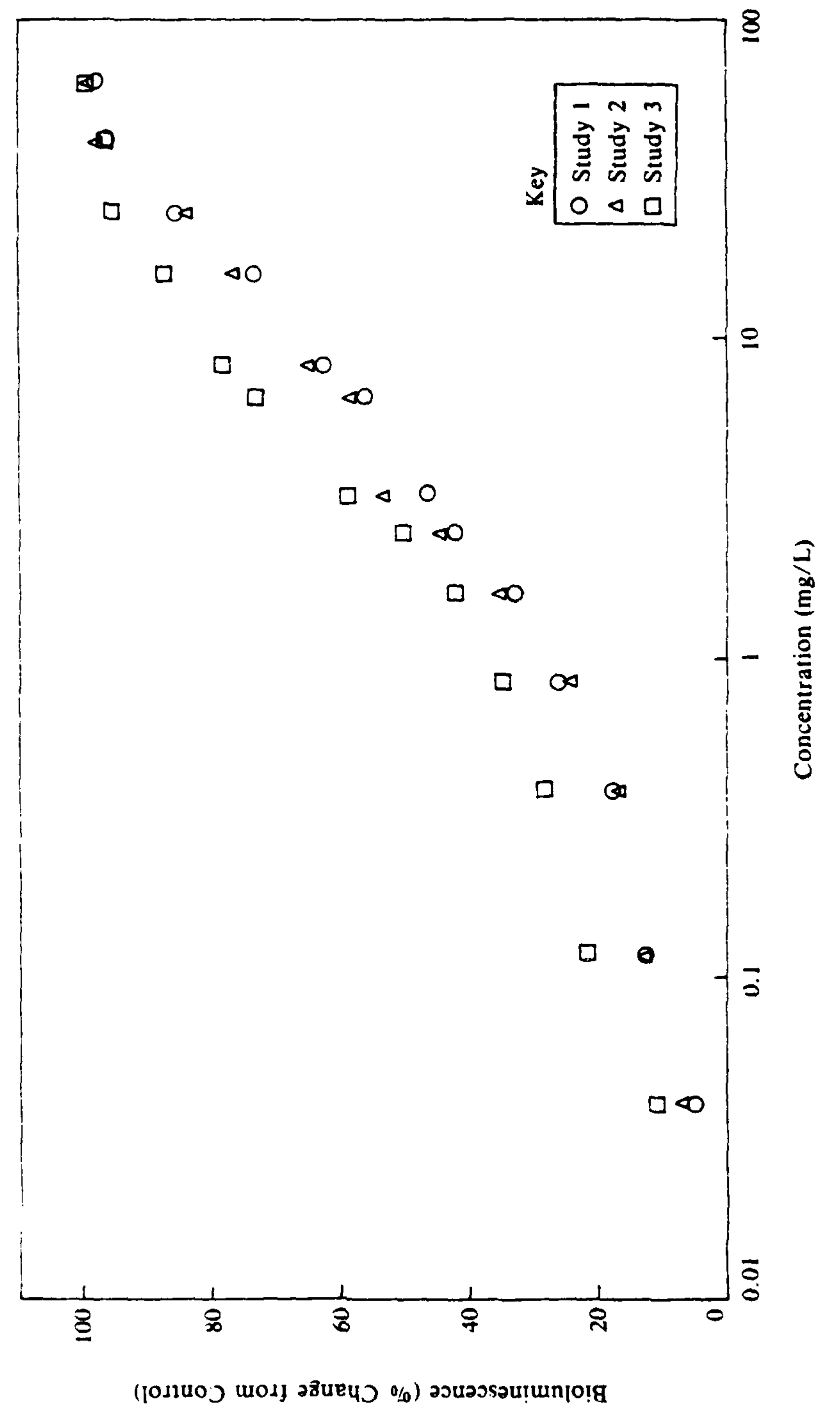

Figure 1. Bioluminescent Response of *B. harveyi* to Selenium Dioxide.

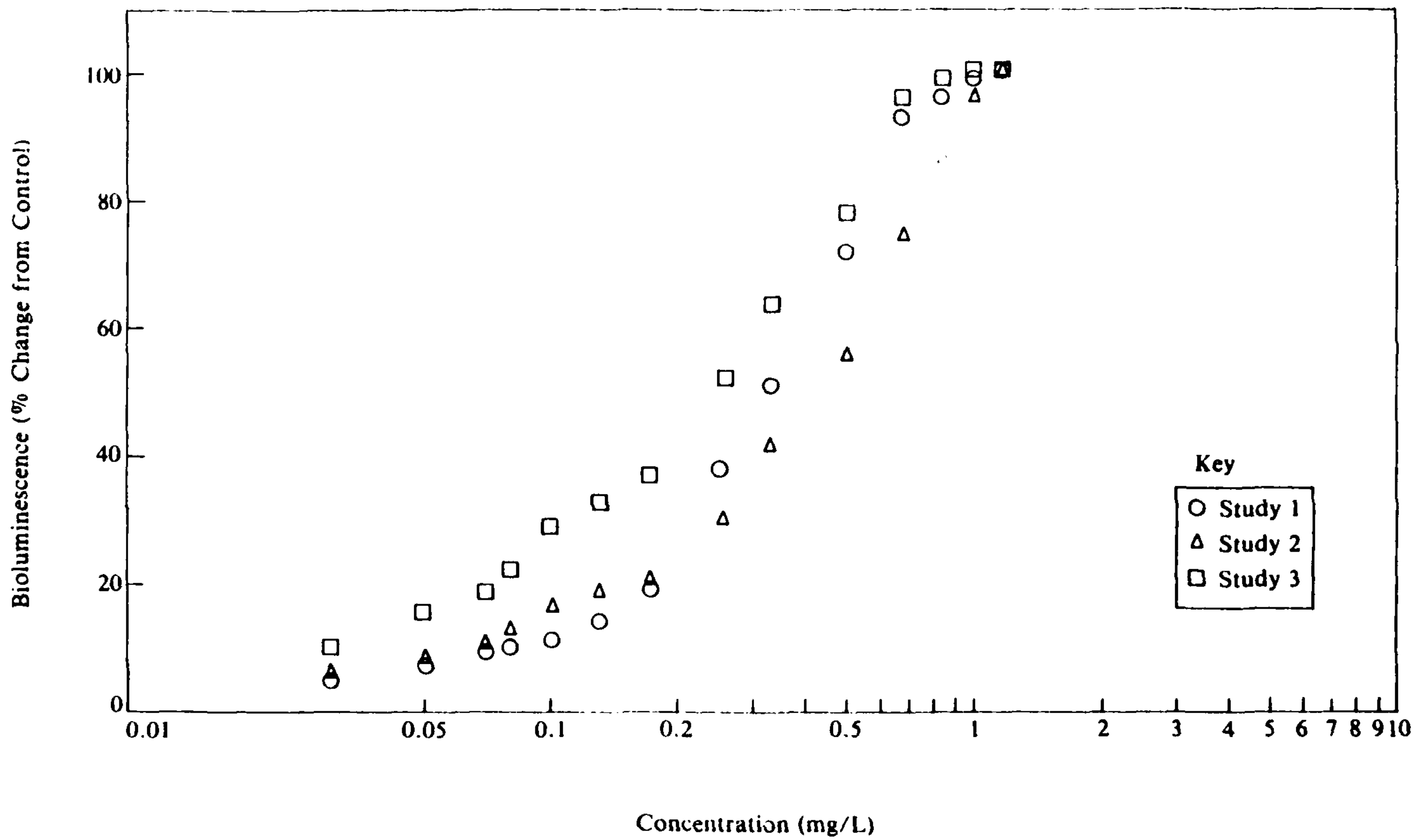

Figure 2. Bioluminescent Response of *B. harveyi* to Mercuric Chloride.

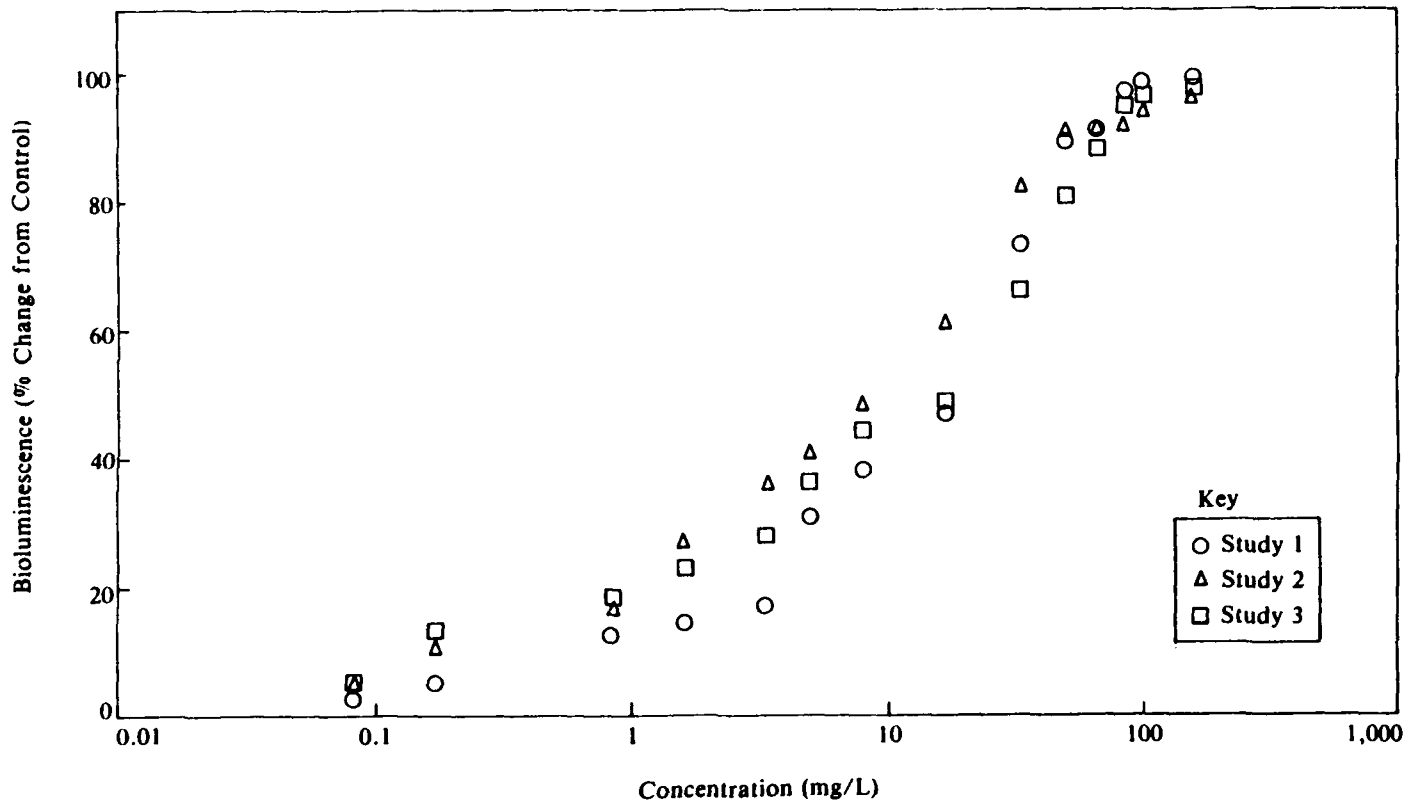

Figure 3. **Bioluminescent Response of *B. harveyi* to Cadmium Chloride.**

respectively. Each data point on the graphs represents the mean of six replicates. Three dose-response studies were conducted for each chemical on three separate days. An EC_{50} value was estimated by linear regression for each dose-response curve.

The mean EC_{50} values and their corresponding estimates of slope, y-intercept at x = 1, and correlation coefficient for the fitted regression lines are shown in Table 2 for each of the three metals. As determined by the mean EC_{50} values, mercuric chloride appears to be the most potent of the three metals tested. Selenium dioxide is slightly less potent than mercuric chloride, and cadmium chloride is the least potent. A t-test[8] was used to determine whether the above stated conclusions drawn from a visual inspection of the EC_{50} values could be statistically verified. The t-tests were performed in the log scale to stabilize the variances of the individual chemicals. The results are shown in Table 3. Statistically significant differences were detected in the potency of the three metals at or below the 0.01 level. Thus, it was confirmed that mercuric chloride was the most potent, selenium dioxide was slightly less potent than mercuric chloride, and cadmium chloride was the least potent in the photobacterial test system.

TABLE 2. ESTIMATES OF THE EC_{50} FOR SINGLE METAL STUDIES

Study No.	EC_{50} (mg/L)	Slope	Y-intercept (X = 1)	r^2
		Selenium Dioxide		
1	5.15	1.50	42.29	0.88
2	5.53	2.14	38.19	0.80
3	2.75	5.66	34.43	0.92
	$\bar{X}$ = 4.48			
		Mercuric Chloride		
1	0.35	141.80	0.45	0.97
2	0.44	91.36	10.16	0.95
3	0.24	111.01	22.84	0.95
	$\bar{X}$ = 0.34			
		Cadmium Chloride		
1	20.25	1.43	21.11	0.95
2	12.13	1.62	30.25	0.92
3	19.87	0.80	34.03	0.93
	$\bar{X}$ = 17.42			

TABLE 3. DIFFERENCES IN THE MEAN EC_{50} VALUES OF THREE METALS

Compounds	Mean EC_{50} (mg/L)	Result of t-test
Selenium Dioxide	4.48	$p < 0.01$
vs.		
Mercuric Chloride	0.34	
Selenium Dioxide	4.48	$p < 0.01$
vs.		
Cadmium Chloride	17.09	
Mercuric Chloride	0.34	$p < 0.01$
vs.		
Cadmium Chloride	17.09	

Paired Metal Studies

The dose-response curves were plotted with concentration on the log scale as shown in Figures 4 through 9. Each figure shows the results of testing paired combinations of the three metals in three separate studies. The results of a set of six replicates, in which bacteria were exposed to only the metal that was fixed in concentration, are shown at the lower right corner of Figures 4 through 9 (shaded symbols). Although the fit of the linear regression lines used to estimate the EC_{10}, from the single chemical dose-response curves shown in Figures 1, 2, and 3, displayed low correlation coefficients (data not shown), these estimates were quite accurate as judged by the three mean values of the chemical tested alone at a fixed concentration (shaded symbols). A response of approximately 10% is shown by the shaded symbol for each study (Figures 4-9).

An EC_{50} was computed for each paired combination of metals tested. The results of the linear regression analyses are shown in Table 4. The correlation coefficients (r^2 values) ranged from 0.83 to 0.96. The three EC_{50} values from each study were then used to compute a mean EC_{50} value for the single chemical studies as well as for the paired combination studies. These data are shown in Table 5a. The metals tested are shown to the left and their respective mean EC_{50} and standard deviations are shown to the right. It was of interest to determine whether the presence of a fixed level of one chemical at its estimated EC_{10} concentration would affect the dose-response curve of the second chemical when tested together.

Thus, the mean EC_{50} values derived from a paired combination of metals were compared with the mean EC_{50} values of the single metal studies as shown in Table 5b. The data were statistically evaluated by the t-test. Table 5b

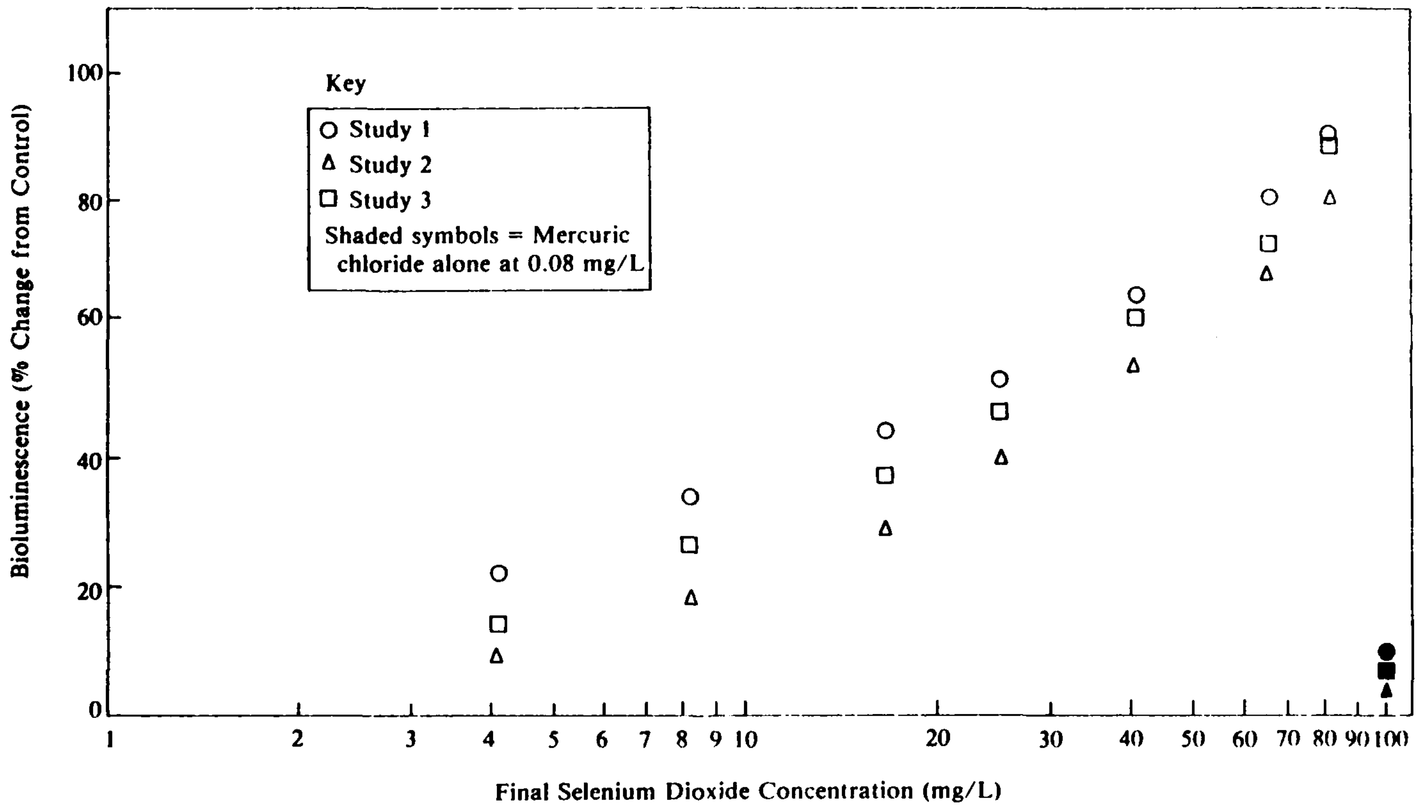

Figure 4. Response of *B. harveyi* to a Fixed Concentration of Mercuric Chloride (0.08 mg/L) and Different Concentrations of Selenium Dioxide.

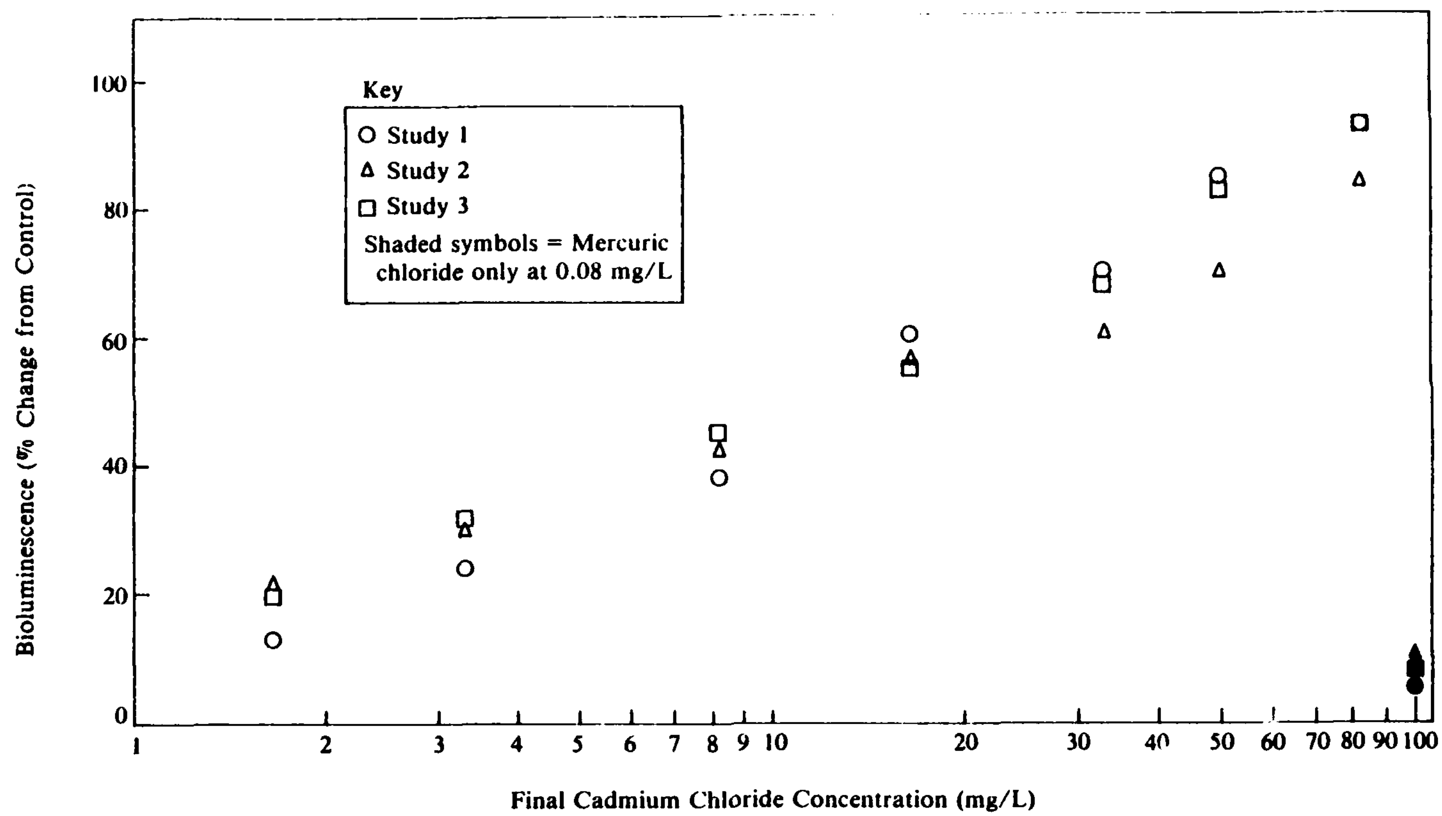

Figure 5. Response of *B. harveyi* to a Fixed Concentration of Mercuric Chloride (0.08 mg/L) and Different Concentrations of Cadmium Chloride.

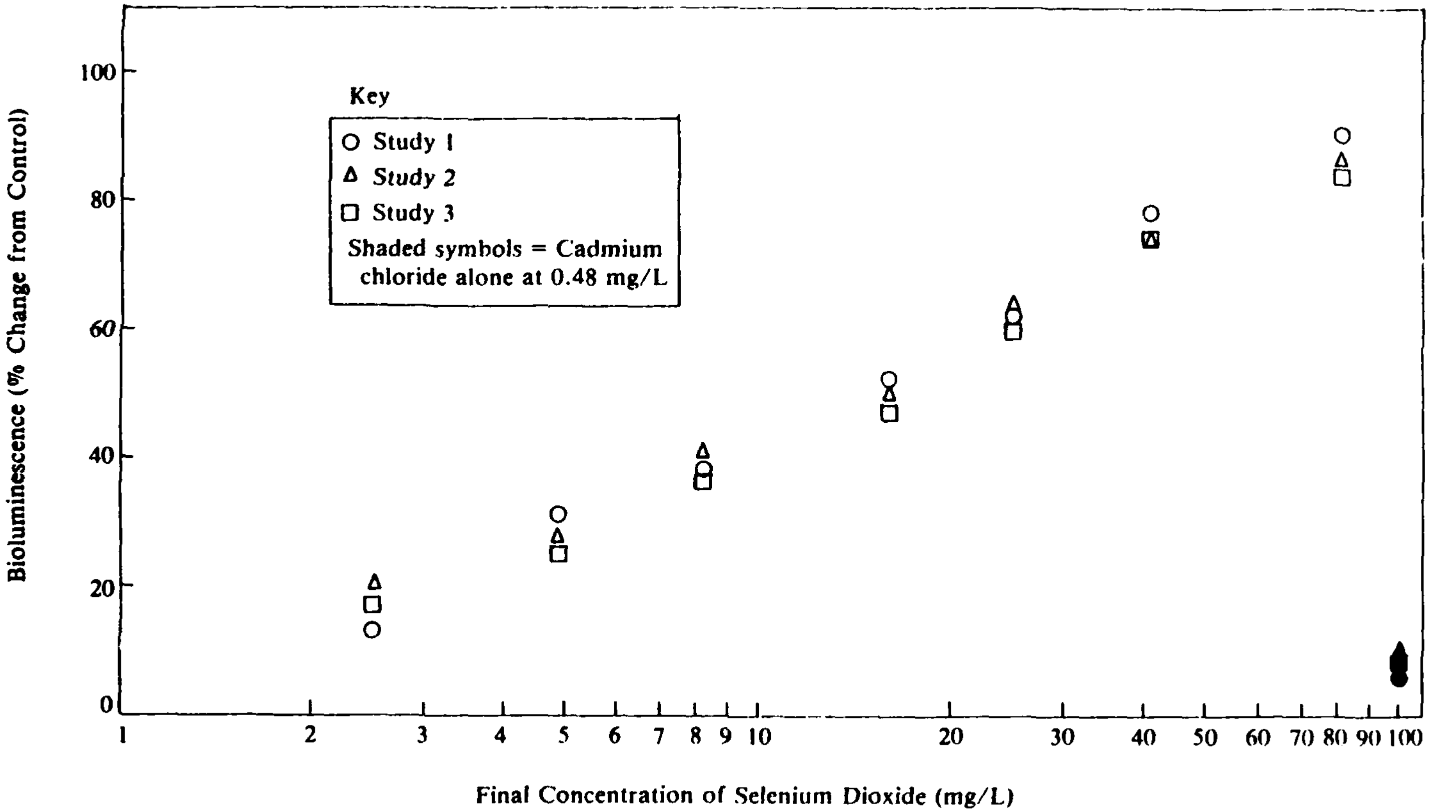

Figure 6. Response of *B. harveyi* to a Fixed Concentration of Cadmium Chloride (0.48 mg/L) and Different Concentrations of Selenium Dioxide.

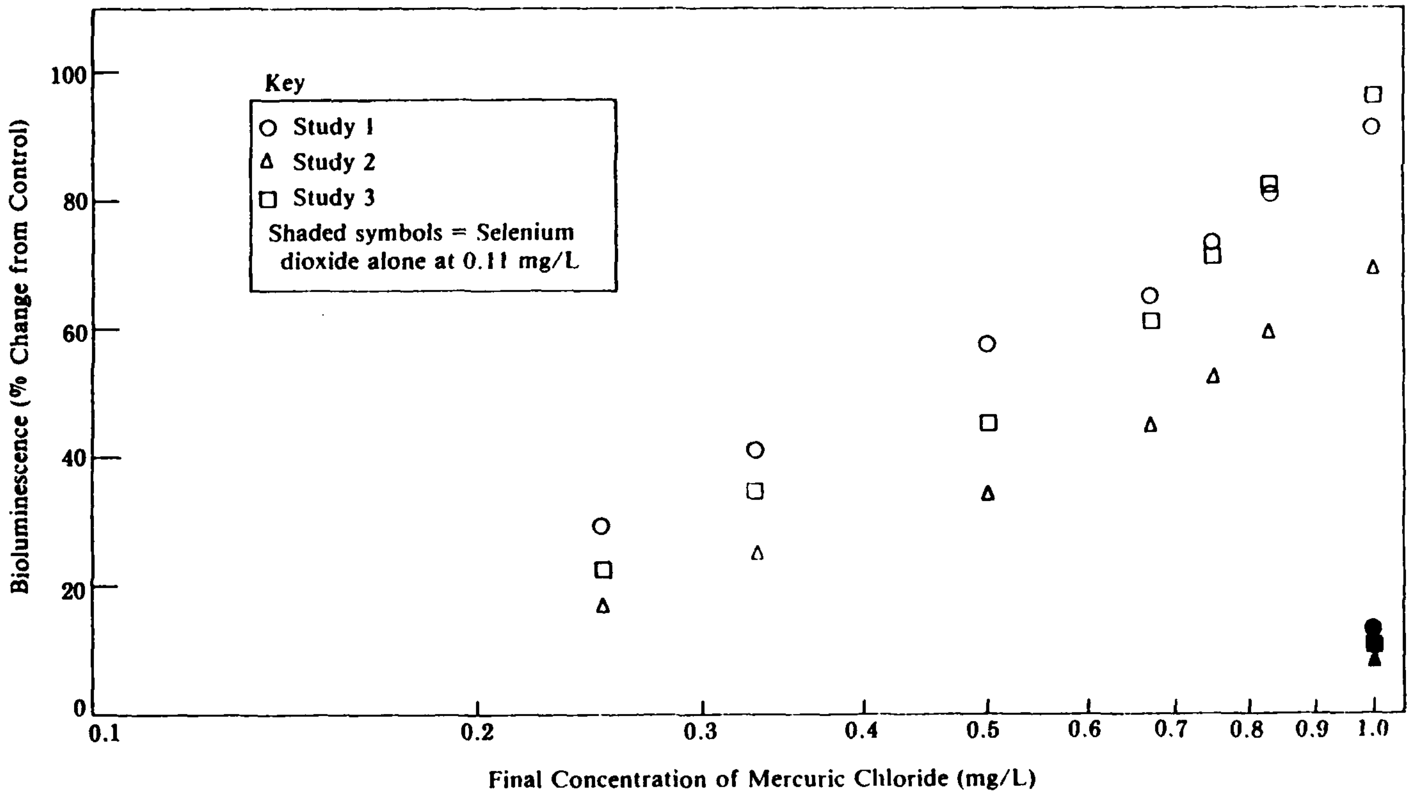

Figure 7. Response of *B. harveyi* to a Fixed Concentration of Selenium Dioxide (0.11 mg/L) and Different Concentrations of Mercuric Chloride.

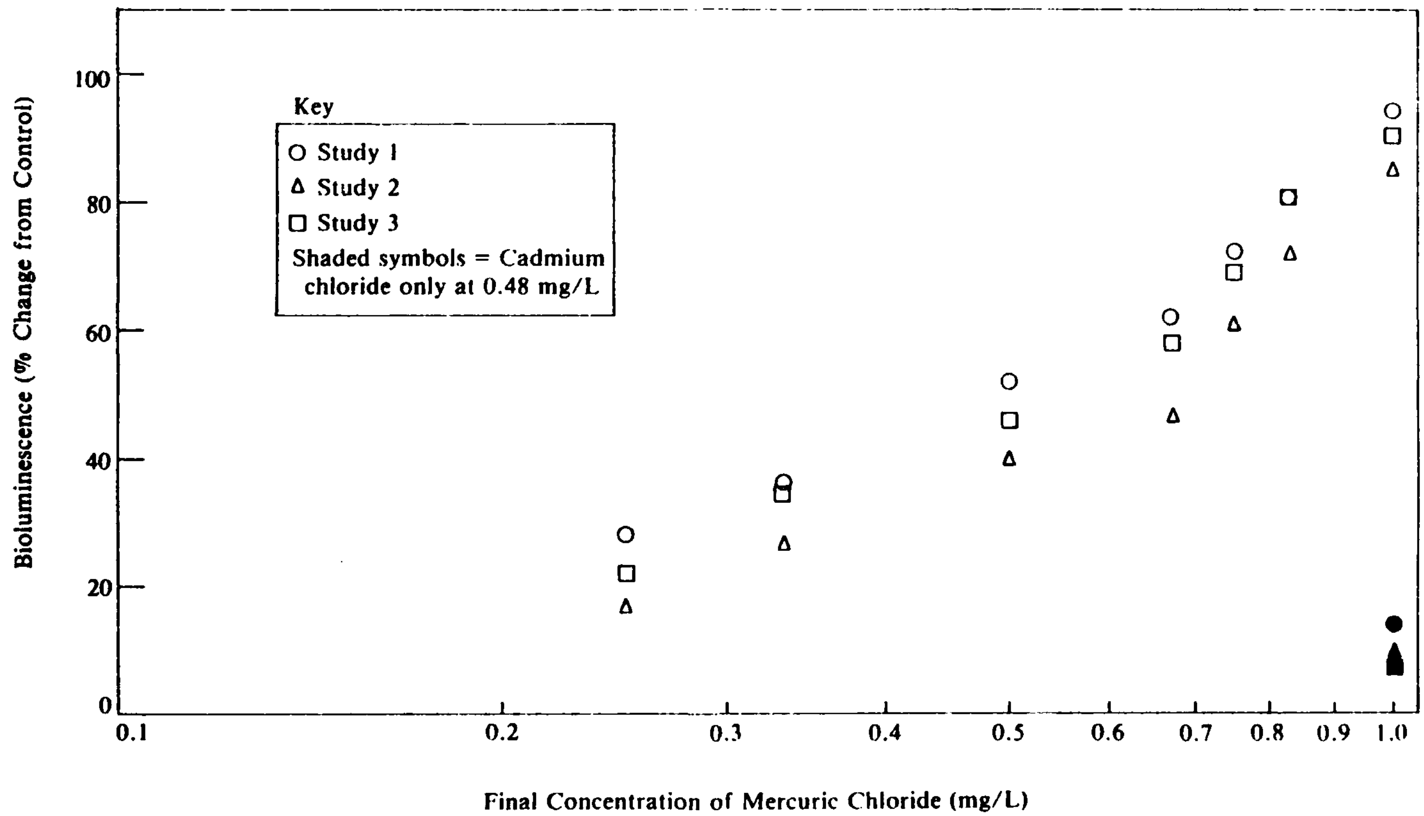

Figure 8. Response of *B. harveyi* to a Fixed Concentration of Cadmium Chloride (0.48 mg/L) and Different Concentrations of Mercuric Chloride.

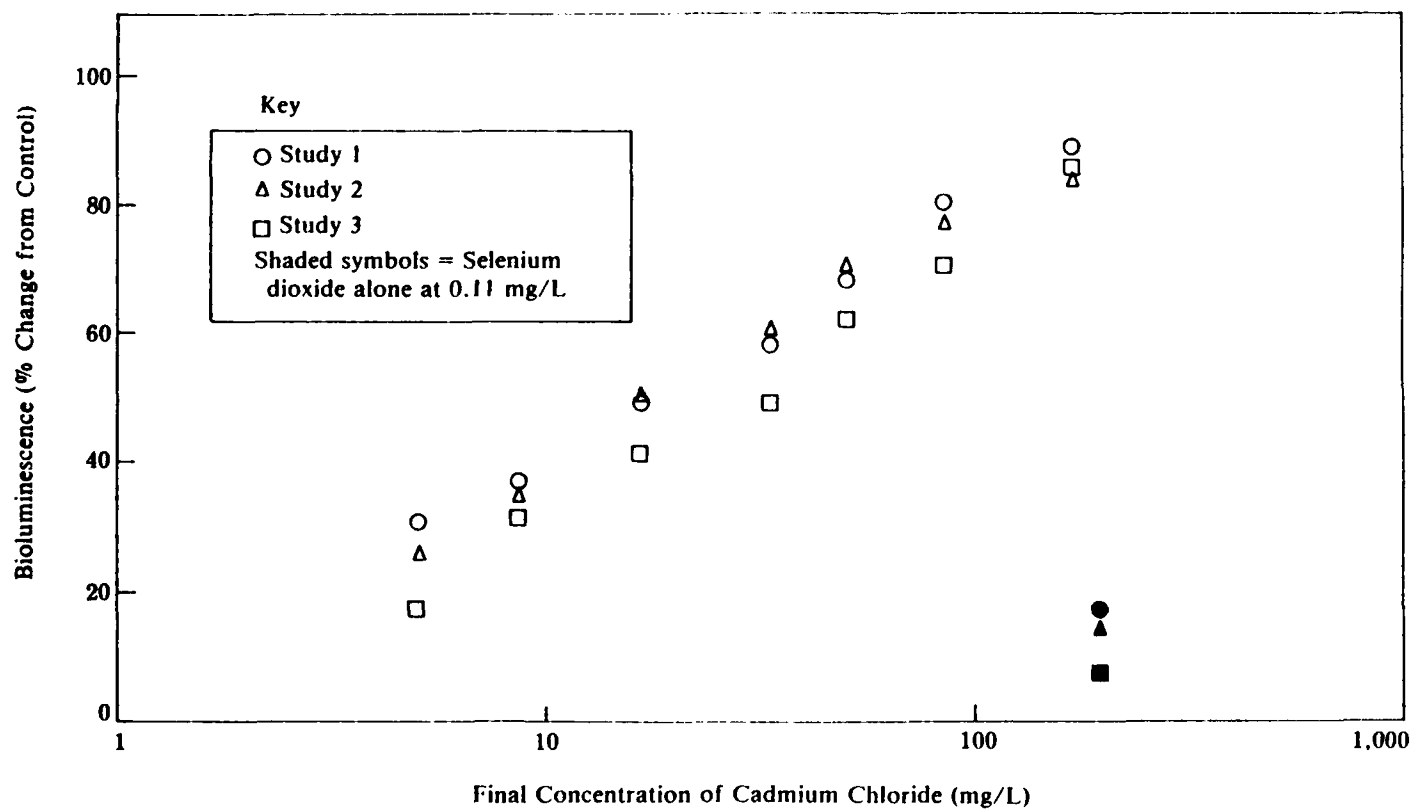

Figure 9. Response of *B. harveyi* to a Fixed Concentration of Selenium Dioxide (0.11 mg/L) and Different Concentrations of Cadmium Chloride.

TABLE 4. LINEAR REGRESSION ANALYSES OF COMBINATION STUDIES FOR THREE METALS

Metal (Concentration)	Study No.	EC_{50} (mg/l)	Slope	Y-intercept (X = 1)	r^2
Mercuric Chloride (varied)	1	0.53	95.51	0.32	0.90
and	2	0.61	91.31	−5.73	0.86
Cadmium Chloride (fixed)	3	0.56	96.50	−3.73	0.89
Mercuric Chloride (varied)	1	0.46	80.51	12.36	0.91
and	2	0.72	74.49	−3.67	0.93
Selenium Dioxide (fixed)	3	0.53	86.37	4.19	0.94
Selenium Dioxide (varied)	1	13.81	0.66	40.84	0.83
and	2	10.71	0.57	43.95	0.83
Cadmium Chloride (fixed)	3	21.17	1.44	19.54	0.92
Selenium Dioxide (varied)	1	22.65	0.69	34.36	0.94
and	2	40.45	0.73	20.64	0.95
Mercuric Chloride (fixed)	3	30.30	0.72	28.20	0.96
Cadmium Chloride (varied)	1	14.35	1.03	35.26	0.85
and	2	9.55	0.47	45.52	0.90
Mercuric Chloride (fixed)	3	6.91	0.61	45.75	0.90
Cadmium Chloride (varied)	1	26.34	0.61	33.87	0.93
and	2	28.58	0.64	31.62	0.84
Selenium Dioxide (fixed)	3	36.86	0.51	31.13	0.90

TABLE 5. SUMMARY OF MEAN EC_{50} VALUES AND COMPARISON OF RESPONSE BY T-TEST

a. Summary of Mean EC_{50} Values

No.		$\bar{X}$	SD
1	Mercuric Chloride	0.34	0.10
2	Selenium Dioxide	4.48	1.51
3	Cadmium Chloride	17.42	4.58
4	Mercuric Chloride (varied) + Cadmium Chloride (fixed)	0.57	0.04
5	Mercuric Chloride (varied) + Selenium Dioxide (fixed)	0.57	0.13
6	Selenium Dioxide (varied) + Cadmium Chloride (fixed)	15.23	5.37
7	Selenium Dioxide (varied) + Mercuric Chloride (fixed)	31.13	5.58
8	Cadmium Chloride (varied) + Mercuric Chloride (fixed)	10.27	3.77
9	Cadmium Chloride (varied) + Selenium Dioxide (fixed)	30.59	5.54

b. Comparison of Response by t-test

Comparisons	p	Type of Interaction
1 vs. 4	< 0.05	Protection
1 vs. 5	N.S.*	No change
2 vs. 6	< 0.02	Protection
2 vs. 7	< 0.01	Protection
3 vs. 8	N.S.	No change
3 vs. 9	< 0.05	Protection

*Not significant.

summarizes the comparison of the EC_{50} values estimated from dose-response curves of single metal studies to those from paired metal studies. The results revealed that the presence of cadmium chloride at approximately its EC_{10} concentration reduced the potency of mercuric chloride and selenium dioxide. When mercuric chloride was added at a fixed concentration (EC_{10}), the potency of selenium dioxide, but not that of cadmium chloride, was reduced. The presence of selenium dioxide at its EC_{10} concentration reduced the potency of cadmium chloride, but not mercuric chloride.

In each combination of the three metals, the metal held at a fixed (EC_{10}) concentration exerted an antagonistic effect or it did not influence the potency of the second metal. None of the combinations resulted in an increase in potency. The presence of mercuric chloride at a fixed concentration appeared to cause a slight decrease in the EC_{50} of cadmium chloride. However, this apparent difference was not statistically significant. Thus, no synergistic effects in potency were observed in this study.

DISCUSSION

The dose-response curves for each of the three metals tested separately were plotted as percent response versus the logarithm of the concentration. The dose-response curves exhibited a linear region surrounding the EC_{50} and, thus, no further data conversion was considered necessary. The EC_{50} values from the single metal studies were compared among themselves and with those from the paired combination of metals studies to assess differences in potency.

The results of this study clearly indicated that cadmium chloride was the least potent of the three metals tested, that selenium dioxide was approximately 4 times more potent than cadmium chloride, and that mercuric chloride was slightly more than 13 times as potent as selenium dioxide. These conclusions are drawn from the differences among the mean EC_{50} values as shown in Table 5a. Since the dose-response curves for the different metals are not parallel as determined by the marked differences in their respective slopes, the quantification of relative potency presented above applies only to the region of the dose-response curve at the EC_{50} estimates. A comparison of the relative potency of the three metals in this photobacterial test system with estimates of their relative toxicity in higher species is difficult. Although an abundance of toxicity data has been reported for each metal, a review of the literature did not reveal studies indicating the relative toxicity of mercuric chloride, cadmium chloride, and selenium dioxide in a single species and sex, using the same endpoint, and under the same controlled experimental conditions. Thus, quantitative comparisons of the results of the photobacterial response data could not be made with other species.

The results of the studies in which different pairs of the three metals were tested are extremely interesting. The predominant results were the protective action exhibited by certain combinations. Each of the specific combinations is discussed here in greater detail.

Selenium dioxide when presented at approximately its EC_{10} concentration resulted in a protective action against cadmium chloride effects but not against mercuric chloride effects. However, it should be noted that selenium dioxide did produce nearly a twofold decrease in the potency of mercuric chloride (Table 5a), but this difference was not statistically significant (Table 5b). The reports in the literature substantiate in other species our observed effect of selenium dioxide on cadmium chloride. Selenium dioxide induced reduction of testicular damage, and other acute effects of cadmium toxicity have been reported by Holmberg and Ferm,[9] Parizek,[3] Mason and Young,[10] and Gunn et al.[11] The literature does not support our findings of no effect of selenium dioxide on mercuric chloride potency. Parizek and Ostadolova[12] first showed in rats that sodium selenite has a protective effect against renal necrosis induced by simultaneous injection of mercuric chloride. Sukra et al.[13] demonstrated that low levels of selenium improved the survival rate of chick embryo to mercury toxicity.

Our inability to show a statistically significant difference in view of a twofold difference in the effect of selenium on the dose-response curve of mercuric chloride may have been due to the relatively large variance of the mean EC_{50} of the paired metal studies; a protective action might have been found if a larger number of studies were conducted or if the variability between studies were reduced by other means.

Cadmium chloride presented at a low (EC_{10}) fixed dose clearly reduced the potency of both mercuric chloride and selenium dioxide (Table 5). These findings are consistent with those reported in the literature. Hill[14] reported that dietary cadmium partially reversed the toxicity of selenium in chicks. Magos and Webb[15] showed that pretreatment of rats with cadmium protects them against the nephrotoxic effect of inorganic mercury.

Lastly, mercuric chloride when presented at its EC_{10} concentration reduced the potency of selenium but did not affect the dose-response curve of cadmium chloride. The ability of mercury to reduce the toxic effects of selenium has been demonstrated by, among others, Parizek et al.,[12] Hill,[14] and Taylor et al.[16]

Thus, the results of the tests using paired combinations of the three metals are consistent with the findings in other species with two exceptions. The two exceptions noted in our studies were the inability of selenium dioxide to affect the mercuric chloride EC_{50} and the inability of mercuric chloride to affect the cadmium chloride EC_{50}. The effect of a fixed dose of selenium dioxide on the potency of mercuric chloride seems to indicate a protective action. There is nearly a twofold increase in the EC_{50} value as shown in Table 5a. Thus, it is anticipated that a statistically significant difference can be detected if appropriate measures are taken in future studies to reduce the variance of the mean EC_{50}s.

The effect of a fixed dose of mercuric chloride on the dose-response curve of cadmium chloride is more interesting. The presence of mercuric chloride seemed to cause a shift in the dose-response curve toward increased

potency. Although not statistically significant, this combination was the only one indicating a tendency toward increasing potency.

These results are most promising because the photobacterial system seems to be predictive of the interactive effects of the three metals observed in higher species. The conclusions drawn from this study are limited by the design of the study. Recall that one metal was presented at a fixed concentration while the second was varied in dose. It would be of interest to determine what the response would be if higher doses of the first metal were also tested. Would the antagonistic response continue or would there be an additive or synergistic response? The specific type of response may very well be dose-dependent. The prospects of additive or synergistic events occurring are suggested by Schubert et al.[17]

A study in which both metals are varied is now in progress at this laboratory. Such a study will more clearly delineate the interactions of the three metals herein discussed.

REFERENCES

1. Muul, I., A.F. Hegyeli, and J.C. Dacre. 1976. Toxicological testing dilemma. Science 193:834.

2. Shiotsuka, R.N., A.F. Hegyeli, P.H. Gibbs, and B. Siggins. 1980. A Short-term Toxicity Screening Test Using Photobacteria. A Feasibility Study. Technical Report 8002, AD A087035. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD.

3. Parizek, J. 1978. Interactions between selenium compounds and those of mercury or cadmium. Environ. Health Perspect. 25:53.

4. Berlin, M. 1978. Interaction between selenium and inorganic mercury. Environ. Health Perspect. 25:67.

5. Magos, L. 1976. The role of synergism and antagonism in the toxicity of metals. In G.F. Nordberg, ed. Effects and Dose-Response Relationships of Toxic Metals. Elsevier, Amsterdam. pp. 491-497.

6. Parizek, J. 1976. Interrelationships among trace elements. In G.F. Nordberg, ed. Effects and Dose-Response Relationships of Toxic Metals. Elsevier, Amsterdam. p. 498.

7. Groth, D.H., L. Stettler, and G. Mackey. 1976. Interactions of mercury, cadmium, selenium, tellurium, arsenic and beryllium. In G.F. Nordberg, ed. Effects and Dose-Response Relationships of Toxic Metals. Elsevier, Amsterdam. p. 527.

8. Snedecor, G. and C. Williams. 1962. Statistical Methods, 4th ed. Iowa State University Press, Ames, IA.

9. Holmberg, R.E., Jr. and V.H. Ferm. 1969. Interrelationships of selenium, cadmium and arsenic in mammalian teratogenesis. Arch. Environ. Health 18:873.

10. Mason, K.E. and J.O. Young. 1967. Effectiveness of selenium and zinc in protecting against cadmium-induced injury of rat testis. In O.H. Muth, ed. Symposium: Selenium in Biomedicine. Avi, Westport, CT. p. 383.

11. Gunn, S.A., T.C. Gould, and W.A.D. Anderson. 1966. Protective effect of thiol compounds against cadmium-induced vascular damage to testis. Proc. Soc. Exp. Biol. Med. 122:1036.

12. Parizek, J., I. Ostadolova, J. Kalouskova, A. Babicky, L. Pavlik, and B. Bibr. 1971. Effect of mercuric compounds on the maternal transmission of selenium in the pregnant and lactating rat. J. Reprod. 25:157.

13. Sukra, Y., S. Sastrohadinoto, and H. Haeruman, Jr. 1976. Effect of selenium and mercury on survival of chick embryos. Poultry Sci. 55:1423.

14. Hill, C.H. 1974. Reversal of selenium toxicity in chicks by mercury, copper and cadmium. J. Nutr. 104:593.

15. Magos, L. and M. Webb. 1978. Theoretical and practical considerations on the problem of metal-metal interaction. Environ. Health Perspect. 25:151.

16. Taylor, T.J., F. Rieders, and J.J. Kocsis. 1978. Toxicological interactions of mercury and selenium. Toxicol. Appl. Pharmacol. 45(1):347.

17. Schubert, J., E.J. Riley, and S.A. Tyler. 1978. Combined effects in toxicology - A rapid systematic testing procedure: cadmium, mercury and lead. J. Toxicol. Environ. Health 4:763.

APPENDIX A

RAW DATA
FROM SINGLE TEST CHEMICAL STUDIES

Raw data from bioluminescence measurements of photobacterial cultures exposed to different concentrations of a single test chemical (mercuric chloride, cadmium chloride, or selenium dioxide) and a control culture, the latter given an equivalent volume of water with no test chemical.

Luminescence is expressed as relative intensity values based on the preset scale of the photometer (range 0 to 100).

TABLE A-1. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO MERCURIC CHLORIDE (STUDY 1)

	Final Concentration of Mercuric Chloride (mg/L)														
Sample	Control	0.03	0.05	0.07	0.08	0.10	0.13	0.17	0.25	0.33	0.50	0.67	0.84	1.00	1.17
1	87	84	81	79	79	82	75	71	55	45	26	2.9	3.5	0.0	0.0
2	88	86	83	81	85	80	73	72	52	42	28	3.8	2.6	0.3	0.0
3	92	83	85	83	79	79	77	68	49	36	21	6.4	3.8	0.4	0.0
4	90	86	80	79	78	81	76	72	58	47	23	5.2	4.9	0.8	0.0
5	88	85	81	79	76	78	78	76	59	46	27	8.3	0.8	0.5	0.0
6	90	84	85	82	83	76	79	72	57	44	--	8.8	4.8	0.6	0.0
Mean	89.2	84.7	82.5	80.5	80.0	79.3	76.3	71.8	55.0	43.3	25.0	5.9	3.4	0.4	0.0

TABLE A-2. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO CADMIUM CHLORIDE (STUDY 1)

	Final Concentration of Cadmium Chloride (mg/L)														
Sample	Control	0.08	0.17	0.84	1.67	3.34	5.01	8.35	16.70	33.40	50.10	66.80	83.50	100.20	116.90
1	69	70	68	60	59	65	53	47	41	15	9.5	6.8	0.9	0.3	0.0
2	68	72	67	58	62	59	49	44	41	22	7.2	6.9	2.9	1.8	0.0
3	76	71	70	66	63	62	50	45	33	24	7.9	7.1	4.6	2.1	1.1
4	77	70	71	68	62	55	52	43	39	19	6.8	6.5	3.5	0.8	0.9
5	75	72	69	63	64	60	48	--	40	20	7.1	--	--	3.1	0.9
6	71	73	68	67	65	58	50	48	39	--	7.8	--	--	1.6	1.1
Mean	72.7	71.3	68.8	63.7	62.5	59.8	50.3	45.4	38.8	20.0	7.7	6.8	3.0	1.6	0.7

TABLE A-3. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO SELENIUM DIOXIDE (STUDY 1)

	Final Concentration of Selenium Dioxide (mg/L)													
Sample	Control	0.04	0.11	0.39	0.84	1.61	2.45	3.29	6.58	8.23	16.42	24.64	41.06	61.60
1	79	73	70	65	60	53	48	42	35	29	22	9.1	2.0	1.9
2	80	78	69	66	60	55	47	43	36	33	22	10	4.5	1.3
3	78	76	68	65	58	55	46	42	38	29	23	12	2.5	1.8
4	82	75	71	68	61	51	48	45	35	27	22	11	3.0	1.0
5	80	77	70	64	58	52	44	44	31	30	21	16	2.6	0.9
6	81	74	68	66	59	55	46	43	35	29	19	14	3.1	2.1
Mean	80.0	75.5	69.3	65.7	59.3	53.5	46.5	43.2	35.0	29.5	21.5	12.0	3.0	1.5

TABLE A-4. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO MERCURIC CHLORIDE (STUDY 2)

	Final Concentration of Mercuric Chloride (mg/L)														
Sample	Control	0.03	0.05	0.07	0.08	0.10	0.13	0.17	0.25	0.33	0.50	0.67	0.84	1.00	1.17
1	86	82	81	78	77	75	66	68	64	49	33	26	16	1.1	0.0
2	85	85	80	75	76	68	69	70	67	48	39	19	10	4.5	0.0
3	86	80	78	81	75	73	69	66	58	50	32	15	9	0.9	0.0
4	86	84	--	69	75	76	78	68	54	47	38	25	15	2.1	0.0
5	81	79	--	83	73	72	68	68	56	55	41	22	8	--	0.0
6	93	--	--	--	70	69	69	72	63	--	45	25	7	--	0.0
Mean	86.2	82.0	79.7	77.2	74.3	72.2	69.8	68.7	60.3	49.8	38.0	22.0	10.8	2.2	0.0

TABLE A-5. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO CADMIUM CHLORIDE (STUDY 2)

	Final Concentration of Cadmium Chloride (mg/L)														
Sample	Control	0.08	0.17	0.84	1.67	3.34	5.01	8.35	16.70	33.40	50.10	66.80	83.50	100.20	116.90
1	60	63	58	58	51	31	36	35	29	9.3	6.1	5.7	6.4	3.9	2.9
2	66	58	58	52	48	44	43	39	21	14	6.3	6.6	5.3	3.5	1.9
3	64	60	60	52	44	43	40	28	28	11	5.9	5.4	5.8	2.9	2.5
4	63	63	62	56	46	46	38	33	25	9	7.3	5.9	4.9	4.8	3.1
5	66	65	54	51	--	44	35	35	23	15	6.4	6.3	5.0	4.3	2.2
6	71	60	56	--	--	--	39	--	26	13	7.8	6.2	5.8	5.5	2.8
Mean	65.0	61.5	58.0	53.8	47.3	41.6	38.5	34.0	25.3	11.9	6.6	6.0	5.5	4.2	2.6

TABLE A-6. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO SELENIUM DIOXIDE (STUDY 2)

	Final Concentration of Selenium Dioxide (mg/L)													
Sample	Control	0.04	0.11	0.39	0.84	1.61	2.45	3.29	6.58	8.23	16.42	24.64	41.06	61.60
1	98	--	--	83	78	66	58	44	39	35	28	21	5.4	0.0
2	97	87	84	77	78	60	56	48	41	39	18	19	2.4	1.1
3	99	83	88	81	69	58	57	42	45	38	26	14	3.2	1.5
4	--	89	--	79	73	64	53	49	43	29	22	14	2.1	1.5
5	--	93	--	87	71	68	53	47	38	37	22	13	1.6	1.2
6	--	95	83	82	--	65	--	48	40	26	23	14	4.5	1.6
Mean	98.0	91.4	85.0	81.5	73.8	63.5	55.4	46.3	41.0	34.0	23.2	15.8	3.2	1.2

TABLE A-7. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO MERCURIC CHLORIDE (STUDY 3)

	Final Concentration of Mercuric Chloride (mg/L)														
Sample	Control	0.03	0.05	0.07	0.08	0.10	0.13	0.17	0.25	0.33	0.50	0.67	0.84	1.00	1.17
1	79	64	65	--	59	49	45	49	38	25	15	0.9	0.3	0.0	0.0
2	77	76	67	63	60	57	49	49	33	31	16	0.6	0.4	0.0	0.0
3	73	62	66	65	60	59	--	51	38	19	14	5.3	0.8	0.0	0.0
4	78	70	59	58	58	56	53	48	39	30	19	6.8	0.2	0.0	0.0
5	--	73	62	61	62	52	55	46	40	32	19	5.1	0.5	0.0	0.0
6	--	68	68	65	60	55	53	45	32	29	17	1.3	0.0	0.0	0.0
Mean	76.8	68.8	64.5	62.4	59.8	54.7	51.0	48.0	36.7	27.7	16.7	3.3	0.4	0.0	0.0

TABLE A-8. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO CADMIUM CHLORIDE (STUDY 3)

	Final Concentration of Cadmium Chloride (mg/L)														
Sample	Control	0.08	0.17	0.84	1.67	3.34	5.01	8.35	16.70	33.40	50.10	66.80	83.50	100.20	116.90
1	73	72	59	58	52	47	45	37	39	26	16	8.5	3.3	2.1	3.2
2	69	65	64	56	58	52	45	39	37	20	12	7.7	2.6	2.4	1.9
3	78	70	65	60	56	55	42	44	35	21	14	6.9	3.0	2.6	1.8
4	65	72	58	63	56	49	46	35	40	23	16	9.3	3.4	2.5	2.2
5	72	64	62	59	54	53	45	43	40	27	10	8.7	6.1	2.1	1.9
6	74	68	69	61	57	55	53	44	33	28	15	11	5.3	2.6	0.8
Mean	71.8	68.5	62.8	59.5	55.5	51.8	46.0	40.3	37.3	24.2	13.8	8.7	4.0	2.4	2.0

TABLE A-9. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO SELENIUM DIOXIDE (STUDY 3)

	Final Concentration of Selenium Dioxide (mg/L)													
Sample	Control	0.04	0.11	0.39	0.84	1.61	2.45	3.29	6.58	8.23	16.42	24.64	41.06	61.60
1	78	69	59	66	53	45	42	35	22	20	8	4.3	1.1	0.4
2	82	73	59	57	58	51	44	35	17	19	15	3.2	5.0	0.4
3	81	69	64	54	54	48	39	37	23	15	10	5.9	1.8	0.6
4	80	70	62	56	51	45	39	28	22	19	17	1.9	0.8	1.6
5	79	72	60	59	51	44	37	29	25	16	9	2.8	3.0	0.8
6	--	75	67	54	53	46	39	33	20	18	9	5.3	1.1	--
Mean	80.0	71.3	61.8	57.7	53.3	46.5	40.0	32.8	21.5	17.8	11.3	3.9	2.1	0.8

APPENDIX B

CONVERTED DATA
FROM SINGLE TEST CHEMICAL STUDIES

Data from studies in Appendix A expressed as percent change from the control mean. The following equation was used for data conversion:

$$\text{Bioluminescence} = 100 - [(\text{raw data}/\bar{x} \text{ of control group}) \times 100]$$

TABLE B-1. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO MERCURIC CHLORIDE (STUDY 1)

Sample	Final Concentration of Mercuric Chloride (mg/L)													
	0.03	0.05	0.07	0.08	0.10	0.13	0.17	0.25	0.33	0.50	0.67	0.84	1.00	1.17
1	5.8	9.2	11.4	11.4	8.1	15.9	20.4	38.3	49.5	70.9	96.7	96.1	100	100
2	3.6	7.0	9.2	4.7	10.3	18.2	19.3	41.7	52.9	68.6	95.7	97.1	99.7	100
3	7.0	4.7	7.0	11.4	11.4	13.7	23.8	45.1	59.6	76.5	92.8	95.7	99.6	100
4	3.6	10.3	11.4	12.6	9.2	14.8	19.3	35.0	47.3	74.2	94.2	94.5	99.1	100
5	4.7	9.2	11.4	14.8	12.6	12.6	14.8	33.9	48.4	69.7	90.7	99.1	99.4	100
6	5.8	4.7	8.1	7.0	14.8	11.4	19.3	36.1	50.7	--	90.1	94.6	99.3	100
Mean	5.1	7.5	9.8	10.3	11.1	14.4	19.5	38.4	51.4	72.0	93.4	96.2	99.5	100

TABLE B-2. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO CADMIUM CHLORIDE (STUDY 1)

	Final Concentration of Cadmium Chloride (mg/L)													
Sample	0.08	0.17	0.84	1.67	3.34	5.01	8.35	16.70	33.40	50.10	66.80	83.50	100.20	116.90
1	3.7	6.5	17.5	18.8	10.6	27.1	35.4	43.6	79.4	86.9	90.6	98.8	99.6	100
2	1.0	7.8	20.2	14.7	18.8	32.6	39.5	43.6	69.7	90.1	90.5	96.0	97.5	100
3	2.3	3.7	9.2	13.3	14.7	31.2	38.1	54.6	67.0	89.1	90.2	93.7	97.1	98.5
4	3.7	2.3	6.5	14.7	24.3	28.5	40.9	46.4	73.9	90.6	91.1	95.2	98.9	98.8
5	2.3	5.1	13.3	12.0	17.5	34.0	--	45.0	72.5	90.2	--	--	95.7	98.8
6	-0.4	6.5	7.8	10.6	20.2	31.2	34.0	46.4	--	89.3	--	--	97.8	98.5
Mean	2.1	5.3	12.4	14.0	17.7	30.8	37.6	46.6	72.5	89.4	90.6	95.9	97.8	99.1

TABLE B-3. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO SELENIUM DIOXIDE (STUDY 1)

	Final Concentration of Selenium Dioxide (mg/L)												
Sample	0.04	0.11	0.39	0.84	1.61	2.45	3.29	6.58	8.23	16.42	24.64	41.06	61.60
1	8.8	12.5	18.8	25.0	33.8	40.0	47.5	56.3	63.8	72.5	88.6	97.5	97.6
2	2.5	13.8	17.5	25.0	31.3	41.3	46.3	55.0	58.8	72.5	87.5	94.4	98.4
3	2.0	15.0	18.8	27.5	31.3	42.5	47.5	52.5	63.8	71.3	85.0	96.9	97.8
4	6.3	11.3	15.0	23.8	36.3	40.0	43.8	56.3	63.3	72.5	86.3	96.3	98.8
5	3.8	12.5	20.0	27.5	35.0	45.0	45.0	61.3	62.5	73.8	80.0	96.8	98.9
6	7.5	15.0	17.5	26.3	31.3	42.5	46.3	56.3	63.8	76.3	82.5	96.1	97.4
Mean	5.2	13.4	17.9	25.9	33.2	41.9	46.1	56.3	62.7	73.2	85.0	96.3	98.2

TABLE B-4. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO MERCURIC CHLORIDE (STUDY 2)

	Final Concentration of Mercuric Chloride (mg/L)													
Sample	0.03	0.05	0.07	0.08	0.10	0.13	0.17	0.25	0.33	0.50	0.67	0.84	1.00	1.17
1	4.8	5.9	9.4	10.6	12.9	23.3	21.1	25.7	43.1	61.7	69.8	81.4	98.7	100
2	1.3	7.1	12.9	11.7	21.1	19.9	18.7	22.2	44.3	54.7	77.9	88.4	94.8	100
3	7.1	9.4	5.9	12.9	15.1	19.9	23.3	32.6	41.9	62.8	82.6	89.5	99.0	100
4	2.4	--	19.9	12.9	11.7	9.4	21.1	37.3	45.4	55.9	71.0	82.6	95.6	100
5	8.3	--	3.6	15.1	16.4	21.1	21.1	35.0	36.1	52.4	74.4	90.7	--	100
6	--	--	--	18.7	19.9	19.9	16.4	26.8	--	47.7	71.0	91.9	--	100
Mean	4.8	7.5	10.3	13.7	16.2	18.9	20.3	29.9	42.1	55.9	74.5	87.4	97.0	100

TABLE B-5. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO CADMIUM CHLORIDE (STUDY 2)

	Final Concentration of Cadmium Chloride (mg/L)													
Sample	0.08	0.17	0.84	1.67	3.34	5.01	8.35	16.70	33.40	50.10	66.80	83.50	100.20	116.90
1	3.1	10.8	10.8	21.5	52.3	44.6	46.2	55.4	85.7	90.6	91.2	90.2	94.0	95.5
2	10.8	10.8	20.0	26.2	32.3	33.8	40.0	67.7	78.5	90.3	89.8	91.8	94.6	97.0
3	7.7	7.7	20.0	32.3	33.8	38.5	56.9	56.9	83.1	91.8	91.7	91.1	95.5	96.2
4	3.8	4.6	13.8	29.2	29.2	41.5	49.2	61.5	86.2	88.8	90.9	92.5	92.6	95.2
5	0.0	16.9	21.5	--	32.3	46.2	46.2	64.6	76.9	90.2	90.3	92.3	93.4	96.6
6	7.7	13.8	--	--	--	40.0	--	60.0	80.0	88.0	90.5	91.1	91.5	95.7
Mean	5.5	10.8	17.2	27.3	36.0	40.8	47.7	61.0	81.7	90.0	90.7	91.5	93.6	96.0

TABLE B-6. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO SELENIUM DIOXIDE (STUDY 2)

	Final Concentration of Selenium Dioxide (mg/L)												
Sample	0.04	0.11	0.39	0.84	1.61	2.45	3.29	6.58	8.23	16.42	24.64	41.06	61.60
1	--	--	15.3	20.4	32.7	40.8	55.1	60.2	64.3	71.4	78.6	94.5	100
2	11.2	14.3	21.4	20.4	38.8	42.9	51.1	58.2	60.2	81.6	80.6	97.6	98.9
3	5.3	10.2	17.3	29.6	40.8	41.8	57.2	54.1	61.2	73.5	85.7	96.7	98.5
4	9.2	--	19.4	25.5	34.7	45.9	50.0	56.1	70.4	77.6	85.7	97.9	98.5
5	5.1	--	11.2	27.6	30.6	45.9	52.1	61.2	62.3	77.6	86.7	98.4	98.8
6	3.1	15.3	16.3	--	33.7	--	51.1	59.2	73.5	76.5	85.7	95.4	98.4
Mean	6.8	13.3	16.8	24.7	35.2	43.5	52.8	58.2	65.3	76.4	83.8	96.8	98.9

TABLE B-7. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO MERCURIC CHLORIDE (STUDY 3)

	Final Concentration of Mercuric Chloride (mg/L)													
Sample	0.03	0.05	0.07	0.08	0.10	0.13	0.17	0.25	0.33	0.50	0.67	0.84	1.00	1.17
1	21.8	20.5	--	27.9	40.1	45.0	40.1	53.5	69.4	81.7	98.9	99.6	100	100
2	4.6	18.1	23.0	26.7	30.3	40.1	40.1	59.7	62.1	80.4	99.3	99.5	100	100
3	24.2	19.3	20.5	26.7	27.9	--	37.7	53.5	76.8	82.9	93.5	99.0	100	100
4	14.4	27.9	29.1	29.1	31.5	35.2	41.3	52.3	63.3	76.8	91.7	99.8	100	100
5	10.8	24.2	25.4	24.2	36.4	32.8	43.8	51.1	60.9	76.8	93.8	99.4	100	100
6	16.9	16.9	20.5	26.7	32.8	35.2	45.0	60.9	64.5	79.2	98.4	100.0	100	100
Mean	15.5	21.2	23.7	26.9	33.2	37.7	41.3	55.2	66.2	79.6	95.9	99.6	100	100

TABLE B-8. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO CADMIUM CHLORIDE (STUDY 3)

	Final Concentration of Cadmium Chloride (mg/L)													
Sample	0.08	0.17	0.84	1.67	3.34	5.01	8.35	16.70	33.40	50.10	66.80	83.50	100.20	116.90
1	0.3	18.3	19.7	28.0	34.9	37.7	48.8	46.0	64.0	77.8	88.2	95.4	97.1	95.6
2	10.0	11.4	22.4	19.7	28.0	37.7	46.0	48.8	72.3	83.4	89.3	96.4	96.7	97.4
3	3.0	10.0	16.9	22.4	23.8	41.8	39.1	51.5	70.9	80.6	90.1	95.8	96.4	97.5
4	0.3	19.7	12.7	22.4	32.1	36.3	51.5	44.6	68.1	77.8	87.1	95.3	96.5	97.0
5	11.4	14.1	18.3	25.2	26.6	37.3	40.4	44.6	62.6	86.1	88.0	91.6	97.1	97.4
6	5.8	4.4	15.5	21.1	23.8	26.6	39.1	54.3	61.2	79.2	84.8	92.7	96.4	98.9
Mean	5.1	13.0	17.6	23.1	28.2	36.2	44.2	48.3	66.5	80.8	87.9	94.5	96.7	97.3

TABLE B-9. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO SELENIUM DIOXIDE (STUDY 3)

	Final Concentration of Selenium Dioxide (mg/L)												
Sample	0.04	0.11	0.39	0.84	1.61	2.45	3.29	6.58	8.23	16.42	24.64	41.06	61.60
1	13.8	26.3	17.5	33.8	43.8	47.5	56.3	72.5	75.0	98.8	94.6	98.6	99.5
2	8.8	26.3	28.8	37.5	36.3	45.0	56.3	78.8	76.3	81.3	96.0	93.8	99.5
3	13.8	20.0	32.5	32.5	40.0	51.3	53.8	71.3	81.3	87.5	92.6	97.8	99.3
4	12.5	22.5	30.0	36.3	43.8	51.3	65.0	72.5	76.3	78.8	97.6	99.0	98.0
5	10.0	25.0	26.3	36.3	45.0	53.8	63.8	68.8	80.0	88.8	96.5	96.3	99.0
6	6.3	16.3	32.5	33.8	42.5	51.3	58.8	75.0	77.5	88.8	93.4	98.6	--
Mean	10.9	22.7	27.9	35.0	41.9	50.0	59.0	73.2	77.7	87.3	95.1	97.4	99.1

APPENDIX C

RAW DATA
FROM COMBINATION OF TEST CHEMICAL STUDIES

Raw data from bioluminescence measurements of photobacterial cultures exposed to a fixed concentration of one chemical and different concentrations of a second. The control group was given only distilled water and the "0" concentration group only the chemical which was fixed in concentration.

Luminescence is expressed as relative intensity values based on the preset scale of the photometer (range = 0 to 100 units).

TABLE C-1. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 1)

		Final Concentration of Cadmium Chloride (mg/L)							
Sample	Control	0	1.67	3.34	8.35	16.70	33.40	50.10	83.50
1	60	55	49	48	39	28	19	10	2.5
2	62	53	52	43	35	23	20	10	4.3
3	58	57	55	46	40	27	21	9	5.7
4	60	54	50	40	33	25	15	11	3.1
5	57	58	51	45	38	20	14	13	3.2
6	55	51	50	47	34	20	15	9	3.9
Mean	58.7	54.7	51.2	44.8	36.5	23.8	17.3	10.3	3.8

TABLE C-2. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 1)

	Final Concentration of Selenium Dioxide (mg/L)								
Sample	Control	0	4.13	8.23	16.42	24.64	41.06	65.70	82.11
1	52	48	43	35	31	29	18	10	4.9
2	50	50	45	30	28	25	21	12	4.5
3	55	47	40	38	33	25	22	22	5.5
4	53	45	39	35	30	23	15	11	7.1
5	52	46	41	32	27	26	19	--	4.5
6	--	49	37	35	28	22	16	8	3.8
Mean	52.4	47.5	40.8	34.2	29.5	25.0	18.5	10.5	5.1

TABLE C-3. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 1)

	Final Concentration of Mercuric Chloride (mg/L)								
Sample	Control	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	40	41	33	33	21	19	12	9.0	2.1
2	45	38	30	28	23	15	10	7.1	0.0
3	48	39	34	27	24	18	13	8.3	1.8
4	46	40	32	26	22	17	15	9.1	4.3
5	46	36	33	28	25	14	10	9.9	5.1
6	44	36	30	28	20	19	14	8.6	2.2
Mean	44.8	38.3	32.0	28.3	22.5	17.0	12.3	8.7	2.6

TABLE C-4. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 1)

	Final Concentration of Selenium Dioxide (mg/L)								
Sample	Control	0	2.45	4.94	8.23	16.42	24.64	41.06	82.11
1	51	49	44	36	35	27	23	12	6.5
2	50	50	45	40	31	25	19	10	5.3
3	50	50	47	38	33	26	22	13	4.8
4	54	47	45	35	30	25	18	19	5.6
5	52	45	47	32	32	23	17	11	3.2
6	54	48	44	34	33	24	20	8	5.1
Mean	51.8	48.2	45.3	35.8	32.3	25.0	19.8	12.2	5.1

TABLE C-5. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 1)

	Final Concentration of Cadmium Chloride (mg/L)								
Sample	Control	0	5.01	8.35	16.70	33.40	50.01	83.50	116.90
1	50	48	41	34	28	21	15	9	7.1
2	58	45	39	36	29	24	17	10	5.4
3	55	44	37	35	28	25	19	9	6.2
4	53	42	35	31	26	23	19	13	5.5
5	56	41	35	33	27	21	18	8	6.1
6	51	45	37	32	28	22	16	12	4.3
Mean	53.8	44.2	37.3	33.5	27.7	22.7	17.3	10.2	5.8

TABLE C-6. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 1)

	Final Concentration of Mercuric Chloride (mg/L)								
Sample	Control	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	57	51	35	37	27	20	19	13	3.1
2	58	51	48	34	25	21	15	18	7.6
3	62	53	44	35	23	22	16	9	5.3
4	59	52	41	32	26	20	17	8	4.4
5	59	50	43	34	22	21	14	12	6.8
6	60	54	41	36	28	21	--	8	--
Mean	59.2	51.8	42.0	34.7	25.2	20.8	16.2	11.3	5.4

TABLE C-7. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 2)

	Final Concentration of Cadmium Chloride (mg/L)								
Sample	Control	0	1.67	3.34	8.35	16.70	33.40	50.10	83.50
1	80	72	60	54	49	36	33	28	10
2	88	76	59	54	47	39	28	25	9
3	75	71	64	57	43	31	31	27	15
4	85	68	66	55	45	36	35	22	16
5	78	72	63	59	42	32	32	--	11
6	76	69	--	57	48	--	30	20	14
Mean	80.3	71.3	62.4	56.0	45.7	34.8	31.5	24.4	12.5

TABLE C-8. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 2)

		Final Concentration of Selenium Dioxide (mg/L)							
Sample	Control	0	4.13	8.23	16.42	24.64	41.06	65.70	82.11
1	--	91	88	79	70	55	46	35	20
2	98	89	85	73	64	58	48	33	15
3	85	90	87	82	63	53	48	28	21
4	97	90	84	75	66	--	43	31	19
5	95	87	85	78	68	60	39	26	17
6	--	--	83	75	--	57	37	30	23
Mean	93.8	89.4	85.3	77.0	66.2	56.6	43.5	30.5	19.2

TABLE C-9. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 2)

	Final Concentration of Mercuric Chloride (mg/L)								
Sample	Control	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	88	80	71	68	53	49	33	27	16
2	91	81	76	63	50	47	39	25	15
3	87	77	74	65	55	49	30	26	8
4	85	79	73	64	50	44	36	21	14
5	88	80	70	60	52	46	32	21	17
6	87	75	--	62	57	43	--	28	9
Mean	87.7	78.7	72.8	63.7	52.8	46.3	34.0	24.7	13.2

TABLE C-10. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF *SELENIUM DIOXIDE* (STUDY 2)

	Final Concentration of Selenium Dioxide (mg/L)								
Sample	Control	0	2.45	4.94	8.23	16.42	24.64	41.06	82.11
1	70	69	60	58	--	42	--	19	13
2	77	70	63	55	48	40	31	17	12
3	81	65	58	53	41	37	28	22	7.5
4	75	71	57	55	46	35	30	20	11
5	75	63	62	51	42	32	27	22	9.8
6	73	68	--	52	--	--	25	16	--
Mean	75.2	67.7	60.0	54.0	44.3	37.2	28.2	19.3	10.7

TABLE C-11. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 2)

	Final Concentration of Cadmium Chloride (mg/L)								
Sample	Control	0	5.01	8.35	16.70	33.40	50.10	83.50	116.90
1	68	59	51	45	38	27	--	15	10
2	70	61	50	43	31	25	20	19	7.5
3	65	58	49	46	35	21	19	--	11
4	--	55	48	42	36	32	23	14	--
5	66	56	52	45	32	30	21	15	11
6	68	--	50	44	--	26	18	12	13
Mean	67.4	57.8	50.0	44.2	34.4	26.8	20.2	15.0	10.5

TABLE C-12. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 2)

	Final Concentration of Mercuric Chloride (mg/L)								
Sample	Control	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	99	88	80	77	69	58	49	40	31
2	98	92	83	75	66	54	51	43	21
3	99	91	81	72	65	50	47	41	27
4	97	87	81	74	62	50	45	41	25
5	98	85	85	71	65	54	45	40	38
6	96	90	80	73	61	53	43	39	32
Mean	97.8	88.8	81.7	73.7	64.7	53.2	46.7	40.7	29.0

TABLE C-13. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 3)

	Final Concentration of Cadmium Chloride (mg/L)								
Sample	Control	0	1.67	3.34	8.35	16.70	33.40	50.10	83.50
1	77	75	67	56	44	38	26	15	5.3
2	81	78	69	58	42	33	20	19	4.8
3	82	78	62	55	47	34	27	16	7.9
4	79	73	60	53	46	34	29	14	6.6
5	82	75	65	59	43	36	23	10	5.8
6	82	74	63	52	--	37	25	9	6.0
Mean	80.5	75.5	64.3	55.5	44.4	35.3	25.0	13.8	6.1

TABLE C-14. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 3)

	Final Concentration of Selenium Dioxide (mg/L)								
Sample	Control	0	4.13	8.23	16.42	24.64	41.06	65.70	82.11
1	88	80	76	60	57	48	35	21	12
2	85	83	75	63	52	44	32	27	10
3	89	84	79	68	55	42	37	22	9.7
4	87	81	74	66	55	49	31	26	11
5	88	80	73	--	53	47	30	22	13
6	86	79	75	64	56	--	36	24	9
Mean	87.2	81.2	75.3	64.2	54.7	46.0	33.5	23.7	10.8

TABLE C-15. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 3)

	Final Concentration of Mercuric Chloride (mg/L)								
Sample	Control	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	80	75	63	55	48	32	27	19	9.8
2	85	77	66	51	44	35	26	17	10.0
3	82	77	62	53	42	36	23	20	7.6
4	86	79	69	57	48	39	25	14	5.3
5	83	77	67	51	45	35	26	11	8.6
6	87	70	63	58	43	35	27	13	8.0
Mean	83.8	75.8	65.0	54.2	45.0	35.3	25.7	15.7	8.2

TABLE C-16. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 3)

	Final Concentration of Selenium Dioxide (mg/L)								
Sample	Control	0	2.45	4.94	8.23	16.42	24.64	41.06	82.11
1	89	80	79	68	56	48	37	20	15
2	91	83	77	68	59	44	34	22	12
3	97	86	69	70	55	49	35	26	10
4	88	80	74	65	53	49	30	23	17
5	84	82	76	67	56	45	39	28	19
6	85	83	76	63	58	48	40	21	15
Mean	89.0	82.3	75.2	66.8	56.2	47.2	35.8	23.3	14.7

TABLE C-17. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 3)

	Final Concentration of Cadmium Chloride (mg/L)								
Sample	Control	0	5.01	8.35	16.70	33.40	50.10	83.50	116.90
1	89	88	--	64	59	44	35	29	12
2	92	85	76	66	55	47	38	30	18
3	91	89	78	58	55	52	33	27	13
4	95	83	80	62	51	43	35	25	11
5	97	85	77	66	50	49	--	27	--
6	--	87	75	66	58	47	36	--	15
Mean	92.8	86.2	77.2	63.7	54.7	47.0	35.4	27.6	13.8

TABLE C-18. LUMINESCENT RESPONSE OF PHOTOBACTERIA TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 3)

	Final Concentration of Mercuric Chloride (mg/L)								
Sample	Control	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	80	73	59	58	45	36	24	12	0.0
2	84	78	63	49	45	31	28	18	3.1
3	79	76	67	57	47	33	25	15	2.6
4	82	73	67	55	49	32	25	16	5.4
5	86	72	65	55	43	32	23	11	3.8
6	88	70	67	53	47	31	20	19	4.7
Mean	83.2	73.7	64.7	54.5	46.0	32.5	24.2	15.2	3.3

APPENDIX D

CONVERTED DATA
FROM COMBINATION OF TEST CHEMICAL STUDIES

Data from Appendix C expressed as percent change from the control mean. The following equation was used for data conversion:

$$\text{Bioluminescence} = 100 - [(\text{raw data}/\bar{x} \text{ of control group}) \times 100]$$

TABLE D-1. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 1)

	Final Concentration of Cadmium Chloride (mg/L)							
Sample	0	1.67	3.34	8.35	16.70	33.40	50.10	83.50
1	6.3	16.5	18.2	33.6	52.3	67.6	83.0	95.7
2	9.7	11.4	26.7	40.4	60.8	65.9	83.0	92.7
3	2.9	6.3	21.6	31.9	54.0	64.2	84.7	90.3
4	8.0	14.8	31.9	43.8	57.4	74.4	94.7	90.3
5	1.2	13.1	23.3	35.3	65.9	76.1	77.9	94.5
6	13.1	14.8	19.9	42.1	65.9	74.4	84.7	93.4
Mean	6.9	12.8	23.6	37.9	59.4	70.4	84.7	92.8

TABLE D-2. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 1)

	Final Concentration of Selenium Dioxide (mg/L)							
Sample	0	4.13	8.23	16.42	24.64	41.06	65.70	82.11
1	8.4	17.9	32.2	40.8	44.7	65.6	80.9	90.6
2	4.6	14.1	42.7	46.6	52.3	59.9	70.1	91.4
3	10.3	23.7	27.5	37.0	52.3	58.0	82.8	89.5
4	14.1	25.6	32.2	42.7	56.1	71.4	79.0	86.5
5	12.2	21.8	38.9	48.5	50.4	63.7	--	91.4
6	6.5	29.4	32.2	46.6	58.0	69.5	84.7	92.7
Mean	9.4	22.1	34.3	43.7	52.3	64.7	79.5	90.4

TABLE D-3. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 1)

	Final Concentration of Mercuric Chloride (mg/L)							
Sample	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	8.5	26.3	26.3	53.1	57.6	73.2	79.9	95.3
2	15.2	33.0	37.5	48.7	66.5	77.7	84.2	100.0
3	12.9	24.1	39.7	46.4	59.8	71.0	81.5	96.0
4	10.7	28.6	42.0	50.9	62.1	66.5	79.7	90.4
5	19.6	26.3	37.5	44.2	68.8	77.7	77.9	88.6
6	19.6	33.0	37.5	55.4	57.6	68.8	80.8	95.1
Mean	14.4	28.6	36.8	49.8	62.1	72.5	80.7	94.2

TABLE D-4. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 1)

	Final Concentration of Selenium Dioxide (mg/L)							
Sample	0	2.45	4.94	8.23	16.42	24.64	41.06	82.11
1	5.4	15.1	30.5	32.4	47.9	55.6	76.8	87.5
2	3.5	13.1	22.8	40.2	51.7	63.3	80.7	89.8
3	3.5	9.3	26.6	36.3	49.8	57.5	74.9	90.7
4	9.3	13.1	32.4	42.1	51.7	65.3	63.3	89.2
5	13.1	9.3	38.2	38.2	55.6	67.2	78.8	93.8
6	7.3	15.1	34.4	36.3	53.7	61.4	90.2	90.2
Mean	7.0	12.5	30.8	37.6	51.7	61.7	77.5	90.2

TABLE D-5. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 1)

	Final Concentration of Cadmium Chloride (mg/L)							
Sample	0	5.01	8.35	16.70	33.40	50.10	83.50	116.90
1	10.8	23.8	36.8	48.0	61.0	72.1	83.3	86.8
2	16.4	27.5	33.1	46.1	55.4	68.4	81.4	90.0
3	18.2	31.2	34.9	48.0	53.5	64.7	83.3	84.5
4	21.9	34.9	42.4	51.7	57.2	64.7	75.8	89.8
5	23.8	34.9	38.7	49.8	61.0	66.5	85.1	88.7
6	16.4	31.2	40.5	48.0	59.1	70.3	77.7	92.0
Mean	17.9	30.6	37.7	48.6	57.9	67.8	81.1	88.6

TABLE D-6. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 1)

	Final Concentration of Mercuric Chloride (mg/L)							
Sample	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	13.9	40.9	37.5	54.4	66.2	67.9	78.0	94.8
2	13.9	18.9	42.6	57.8	64.5	74.6	69.6	87.2
3	10.5	25.7	40.9	61.1	62.8	73.0	84.8	91.0
4	12.2	30.7	45.9	56.1	66.2	71.3	86.5	92.6
5	15.5	27.4	42.6	62.8	64.5	76.4	79.7	88.5
6	8.8	30.7	39.2	52.7	64.5	--	86.5	--
Mean	12.5	29.1	41.5	57.5	64.8	72.6	80.9	90.8

TABLE D-7. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 2)

	Final Concentration of Cadmium Chloride (mg/L)							
Sample	0	1.67	3.34	8.35	16.70	33.40	50.10	83.50
1	10.3	25.3	32.8	39.0	55.2	59.0	65.1	87.5
2	5.4	26.5	32.8	41.5	51.4	65.1	68.9	88.8
3	11.6	20.3	29.0	46.5	61.4	61.4	66.4	81.3
4	15.3	17.8	31.5	44.0	55.2	56.4	72.6	80.1
5	10.3	21.5	26.5	48.0	60.1	60.1	--	86.3
6	14.1	--	29.0	40.2	--	62.6	75.1	82.6
Mean	11.2	22.3	30.3	43.2	56.7	60.8	69.6	84.4

TABLE D-8. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 2)

	Final Concentration of Selenium Dioxide (mg/L)							
Sample	0	4.13	8.23	16.42	24.64	41.06	65.70	82.11
1	3.0	6.2	15.8	25.4	41.4	51.0	62.7	78.7
2	5.1	9.4	22.2	31.8	38.2	48.8	64.8	84.0
3	4.1	7.3	12.6	32.8	43.5	48.8	70.1	77.6
4	4.1	10.5	20.0	29.6	--	54.2	67.0	79.7
5	7.3	9.4	16.8	27.5	36.0	58.4	72.3	81.9
6	--	11.5	20.0	--	39.2	60.6	68.0	75.5
Mean	4.7	9.1	17.9	29.4	39.7	53.6	67.5	79.6

TABLE D-9. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 2)

	Final Concentration of Mercuric Chloride (mg/L)							
Sample	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	8.8	19.0	22.5	39.6	44.1	62.4	69.2	81.8
2	7.6	13.3	28.2	43.0	46.4	55.5	71.5	82.9
3	12.2	15.6	25.9	37.3	44.1	65.8	70.4	90.9
4	9.9	16.8	27.0	43.0	49.8	59.0	76.1	84.0
5	8.8	20.2	31.6	40.7	47.5	63.5	76.1	80.6
6	14.5	--	29.3	35.0	51.0	--	68.1	89.7
Mean	10.3	17.0	27.4	39.8	47.2	61.2	71.9	85.0

TABLE D-10. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 2)

	Final Concentration of Selenium Dioxide (mg/L)							
Sample	0	2.45	4.94	8.23	16.42	24.64	41.06	82.11
1	8.2	20.2	22.9	--	44.1	--	74.7	82.7
2	6.9	16.2	26.9	36.2	46.8	58.8	77.4	84.0
3	13.6	22.9	29.5	45.5	50.8	62.8	70.7	90.0
4	5.6	24.2	26.9	38.8	53.5	60.1	73.4	85.4
5	16.2	17.6	32.2	44.1	57.4	64.1	70.7	87.0
6	9.6	--	30.9	--	--	66.8	78.7	--
Mean	10.0	20.2	28.2	41.2	50.5	62.5	74.3	85.8

TABLE D-11. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.02 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 2)

	Final Concentration of Cadmium Chloride (mg/L)							
Sample	0	5.01	8.35	16.70	33.40	50.10	83.50	116.90
1	12.5	24.3	33.2	43.6	59.9	--	77.7	85.2
2	9.5	25.8	36.2	54.0	62.9	70.3	71.8	88.9
3	13.9	27.3	31.8	48.1	68.8	71.8	--	83.7
4	18.4	28.8	37.7	46.6	52.5	65.9	79.2	--
5	16.9	22.8	33.2	52.5	55.5	68.8	77.7	83.7
6	--	25.8	34.7	--	61.4	73.3	82.2	80.7
Mean	14.2	25.8	34.5	49.0	60.2	70.0	77.7	84.4

TABLE D-12. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 2)

	Final Concentration of Mercuric Chloride (mg/L)							
Sample	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	10.0	18.2	21.3	29.4	40.7	49.9	59.1	68.3
2	5.9	15.1	23.3	32.5	44.8	47.8	56.0	78.5
3	7.0	17.2	26.4	33.5	48.9	51.9	58.1	72.4
4	11.0	17.2	24.3	36.6	48.9	54.0	58.1	74.4
5	13.1	13.1	27.4	33.5	44.8	54.0	59.1	61.1
6	8.0	18.2	25.4	37.6	45.8	56.0	60.1	62.3
Mean	9.2	16.5	24.7	33.9	45.7	52.3	58.4	69.5

TABLE D-13. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 3)

	Final Concentration of Cadmium Chloride (mg/L)							
Sample	0	1.67	3.34	8.34	16.70	33.40	50.10	83.50
1	7.1	17.0	30.6	45.5	52.9	67.8	81.4	93.4
2	3.3	14.5	28.1	48.0	59.1	75.2	76.5	94.1
3	3.3	23.2	31.8	41.8	57.9	66.5	80.2	90.2
4	9.5	25.7	34.3	43.0	57.9	64.1	82.7	91.8
5	7.1	19.5	26.9	46.7	55.4	71.5	87.6	92.8
6	8.3	21.9	35.6	--	54.2	69.0	88.8	92.6
Mean	6.4	20.3	31.2	45.0	56.2	69.0	82.9	92.5

TABLE D-14. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF MERCURIC CHLORIDE (0.08 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 3)

	Final Concentration of Selenium Dioxide (mg/L)							
Sample	0	4.13	8.23	16.42	24.64	41.06	65.70	82.11
1	8.3	12.8	31.2	34.6	45.0	59.9	75.9	86.2
2	4.8	14.0	27.8	40.4	49.5	63.3	69.0	88.5
3	3.7	9.4	22.0	36.9	51.8	57.6	74.8	88.9
4	7.1	15.1	24.3	36.9	43.8	64.4	70.2	87.4
5	8.3	16.3	--	39.2	46.1	65.6	74.8	85.1
6	9.4	14.0	26.6	35.8	--	58.7	72.5	89.7
Mean	6.9	13.6	26.4	37.3	47.2	61.6	72.9	87.6

TABLE D-15. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 3)

	Final Concentration of Mercuric Chloride (mg/L)							
Sample	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	10.5	24.8	34.4	42.7	61.8	67.8	77.3	88.3
2	8.1	21.2	39.1	47.5	58.2	69.0	79.7	88.1
3	8.1	26.0	36.8	49.9	57.0	72.6	76.1	90.9
4	5.7	17.7	32.0	42.7	53.5	70.2	83.3	93.7
5	8.1	20.0	39.1	46.3	58.2	69.0	86.9	89.7
6	16.5	24.8	30.8	48.7	58.2	67.8	84.5	90.5
Mean	9.5	22.4	35.4	46.3	57.8	69.4	81.3	90.2

TABLE D-16. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF CADMIUM CHLORIDE (0.48 mg/L) AND VARIABLE CONCENTRATIONS OF SELENIUM DIOXIDE (STUDY 3)

	Final Concentration of Selenium Dioxide (mg/L)							
Sample	0	2.45	4.94	8.23	16.42	24.64	41.06	82.11
1	10.1	11.2	23.6	37.1	46.1	58.4	77.5	83.1
2	6.7	13.5	23.6	33.7	50.6	61.8	75.3	86.5
3	3.4	22.5	21.3	38.2	44.9	60.7	70.8	88.8
4	10.1	16.9	27.0	40.4	44.9	66.3	74.2	80.9
5	7.9	14.6	24.7	37.1	49.4	56.2	68.5	78.7
6	6.7	14.6	29.2	34.8	46.1	55.1	76.4	83.1
Mean	7.5	15.6	24.9	36.9	47.0	59.8	73.8	83.5

TABLE D-17. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF CADMIUM CHLORIDE (STUDY 3)

	Final Concentration of Cadmium Chloride (mg/L)							
Sample	0	5.01	8.35	16.70	33.40	50.10	83.50	116.90
1	5.2	--	31.0	36.4	52.6	62.3	68.8	87.1
2	8.4	18.1	28.9	40.7	49.4	59.1	67.7	80.6
3	4.1	15.9	37.5	40.7	44.4	64.4	70.9	86.0
4	10.6	13.8	33.2	45.0	53.7	62.3	73.1	88.1
5	8.4	17.0	28.9	46.1	47.2	--	70.9	--
6	6.3	19.2	28.9	37.5	49.4	61.2	--	83.8
Mean	7.2	16.8	31.4	41.1	49.5	61.9	70.3	85.1

TABLE D-18. CONVERTED LUMINESCENCE DATA OF PHOTOBACTERIAL RESPONSE TO A FIXED CONCENTRATION OF SELENIUM DIOXIDE (0.03 mg/L) AND VARIABLE CONCENTRATIONS OF MERCURIC CHLORIDE (STUDY 3)

	Final Concentration of Mercuric Chloride (mg/L)							
Sample	0	0.25	0.33	0.50	0.67	0.75	0.84	1.00
1	12.3	29.1	30.3	45.9	56.7	71.2	85.6	100.0
2	6.3	24.3	41.1	45.9	62.7	66.3	78.4	96.3
3	8.7	19.5	31.5	43.5	60.3	70.0	82.0	96.9
4	12.3	19.5	33.9	41.1	61.5	70.0	80.8	93.5
5	13.5	21.9	33.9	48.3	61.5	72.4	86.8	95.4
6	15.9	19.5	36.3	43.5	62.7	76.0	77.2	94.4
Mean	11.5	22.3	34.5	44.7	60.9	71.0	81.8	96.1

DISTRIBUTION LIST

No. of Copies	
5	US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
12	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1	Commandant Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, TX 78234
2	USAMBRDL Technical Library

DATE

FILMED

8